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A STUDY OF FACTORS (EMOTIONAL) RESPONSIBLE FOR CHANGES IN THE PATTERN OF SPONTANEOUS RHYTHMIC FLUCTUATIONS IN THE VOLUME OF THE VASCULAR BED OF THE FINGER TIP[1]

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When rhythmic changes in the volume of fingers (alpha waves), toes, and ears were first studied, the records seemed to fall into two main types, those with large changes and those with small. There was reason to think that these differences corresponded to the psychological type of the subject—a pattern of consistently large changes occurring in persons with volatile temperaments, and of consistently small changes in steady-going, phlegmatic ones. Investigation into the mechanism of these rhythmic changes disclosed the fact that they were independent of fluctuations in blood pressure (1) but were dependent upon changes in the size of the small blood vessels (2). It became clear that these were in turn dependent upon the uninterrupted activity of the sympathetic nervous supply (3). Information recently secured showed, furthermore, that the pattern of changes was not unvarying in an individual, but could be now of one type and later on of another. Such transformations occurred even when external factors such as temperature and humidity were kept constant and disturbing psychological influences originating in the immediate environment were kept at a minimum. Control over all these elements was maintained because of their known importance as stimuli, capable of modifying the basic pattern by inducing periods of vasoconstriction.

Once the detectable environmental influences were stabilized, cause for the lability of patterns must lie, it seemed, in what was going on in the mind of the person under examination. If parallel records could be compared, (*a*) those of the pattern of changes in volume of the finger with (*b*) those attempting to describe what was going on in the subject's mind, a clue to the influence of the latter, (*b*), upon the former, (*a*), might be obtained. Because the task of setting down and analyzing all that was going on in a subject's mind was obviously beyond the scope of this study, one aspect of mental activity was selected as deserving primary consideration. This was the emotional status. The point should be made emphatic that the objective of this study was limited to a comparison between emotional status and the changing pattern of activity in the peripheral blood vessels. The object was to ascertain whether particular patterns recurred repeatedly and could be identified with particular emotional states.

MATERIALS AND METHOD

The apparatus employed to obtain plethysmographic records of the changes in volume of the finger tips was that of Turner (4). Certain alterations described by Neumann (1) were introduced because they provided easy portability and made it possible to obtain records in an environment completely familiar to the subject. The tip of the left index finger only was measured. Its volume was ascertained beforehand. The distance in the photographic system was adjusted so that the records obtained represented effects produced by 5 cc. of finger tip. This device was convenient and allowed direct comparison of records from fingers of different sizes. The subject was recumbent when the records were made and the finger tip was at the level of his heart. Since air-conditioning equipment was not available, the investigations were conducted during the spring when, by selecting comfortable days, the temperatures fell within 5 degrees of 75° F.,

[1] This is the 11th paper reporting the results of studies of the small blood vessels and related subjects.

[2] The Bureau of Medicine and Surgery does not necessarily undertake to endorse views or opinions which are expressed in this paper.

and the relative humidity between 48 and 79 per cent. Records were not made until at least 3 hours after eating. After the plethysmographic cup had been applied to the finger, subjects rested for about 20 or 30 minutes. Then records were taken for 15 minutes on all but a very few occasions when, for one reason or another, the time had to be somewhat curtailed.

Ten males, 8 psychiatrists, a psychologist, and a physiologist, fully acquainted with the program and problem of this investigation, volunteered to act as subjects. They were willing, of course, and trained to report fully such information as was required. Their ages varied from 28 to 39 years, the average being 33 years. A total of 54 records, an average of 5 per person, was obtained over a period of 10 weeks. The apparatus was placed in a room separated by a sound-proof wall from that of the subjects, to avoid an emotional reaction likely to be created by the presence of an operator and the machinery (5).

After each record was obtained, the subject, in response to careful questioning, described his dominant emotion. Special attention was paid to the presence of persistent emotions. In addition, his behavior just before and during the recording was appraised, particularly in terms of restlessness, degree of cooperation and of curiosity about the procedure. Beside his dominant emotion, its type, intensity, onset, course, and cause, his less dominant ones or the presence of a combination of emotions were likewise recorded. The subjects were also asked to describe the more dominant emotions experienced during the preceding 24 hours. An impression of their general physiological status was obtained by inquiring about fatigue, minor somatic complaints, headache, abdominal discomfort, unusual degree of muscular tension, palpitation, and perspiration.

For the purpose of this investigation, certain emotions received the following definitions. Contentment implied a state of moderate happiness without special cause and without awareness of agreeable, somatic association. Elation was taken to imply happiness, but in this case as a reaction to an external or psychologic event or release from anxiety. Beyond a feeling of physical well-being, elation was usually considered as being free from somatic symptoms. Sadness meant mild unhappiness, unalloyed with other emotions, especially anxiety, and presenting no diurnal variation in mood, no decreased physical activity, no retardation of movement or thinking, no accompanying somatic manifestations. Anxiety was fear or apprehension, usually with restlessness and a feeling of muscular tension (6). Other somatic manifestations, such as tremor, palpitation, and axillary perspiration, were recognized as present in anxiety, but the intensity of this emotion was never great enough to permit outspoken accompaniments. Depression meant unhappiness plus an admixture of other affects, such as anxiety or resentment. Resentment itself implied a frequently formed configuration of emotions in which anxiety, unhappiness, anger, and suspicion were present, with their corresponding somatic appearances.

RESULTS

All subjects exhibited continuous rhythmic variations in volume of the finger tip. These fell into 5 main types (2): pulse waves, respiratory waves, and alpha, beta, and gamma waves. The first 2 are dependent on cardiac and respiratory activity; the latter 3 are independent of other recognized physiological phenomena. Alpha waves may be described briefly as the plethysmographic representations of rhythmical somewhat *unequal* variations in the size of a finger or toe. They occur 5 to 8 times a minute. Their size may attain that of pulse waves recorded from the same part. Beta and gamma fluctuations resemble these but are larger and occur less frequently, requiring long, continued recording for their full development. Their meaning has not yet been investigated.

In the present records, the average number of alpha deflections for all subjects was 5.4, as compared with the average 7.9 per minute formerly published (2). This difference is owing presumably to the study of a different group of subjects, and, in part, to lack of uniformity exhibited by these individuals. Such differences will continue to be discovered because the individuals and groups that have been studied are not representative of a freely selected random sample of persons.

Some records were obtained in which almost all of the *alpha* deflections were small; in others, there were many large deflections; and in still others, there was a predominant number of large deflections. The records could accordingly be divided into the customary 3 types (2). In Type I were those which consisted almost entirely of small deflections, with an average size of 13 cu. mm.[3] or less. In Type II, there were fewer small and more large deflections; the averages in the various individual records ranged between 13 and 17.9 cu. mm. Type III records exhibited many large ones, raising the average size above 18 cu. mm. These 3 types are, for the most part, clearly distinguishable on casual perusal of the tracings. The quantitative limitations have been arbitrarily imposed to aid in the classification of borderline records. The important classification

[3] In this paper, changes in volume (alpha deflections and pulse waves) are given in cubic millimeters per 5 cc. of part.

is among the principal types—small, intermediate, and large deflections. There is obviously no essential difference between a record having an average size of 17.9 cu. mm. and one in which the average is 18 cu. mm.

Independent of variations in the size of alpha deflections were variations in the size of the *pulse* waves. These were recorded simultaneously, and on the tracings appeared as minor, rapid fluctuations (equal in rate to the cardiac beat), modulating the slower and larger alpha waves.

There were records in which all the pulse waves

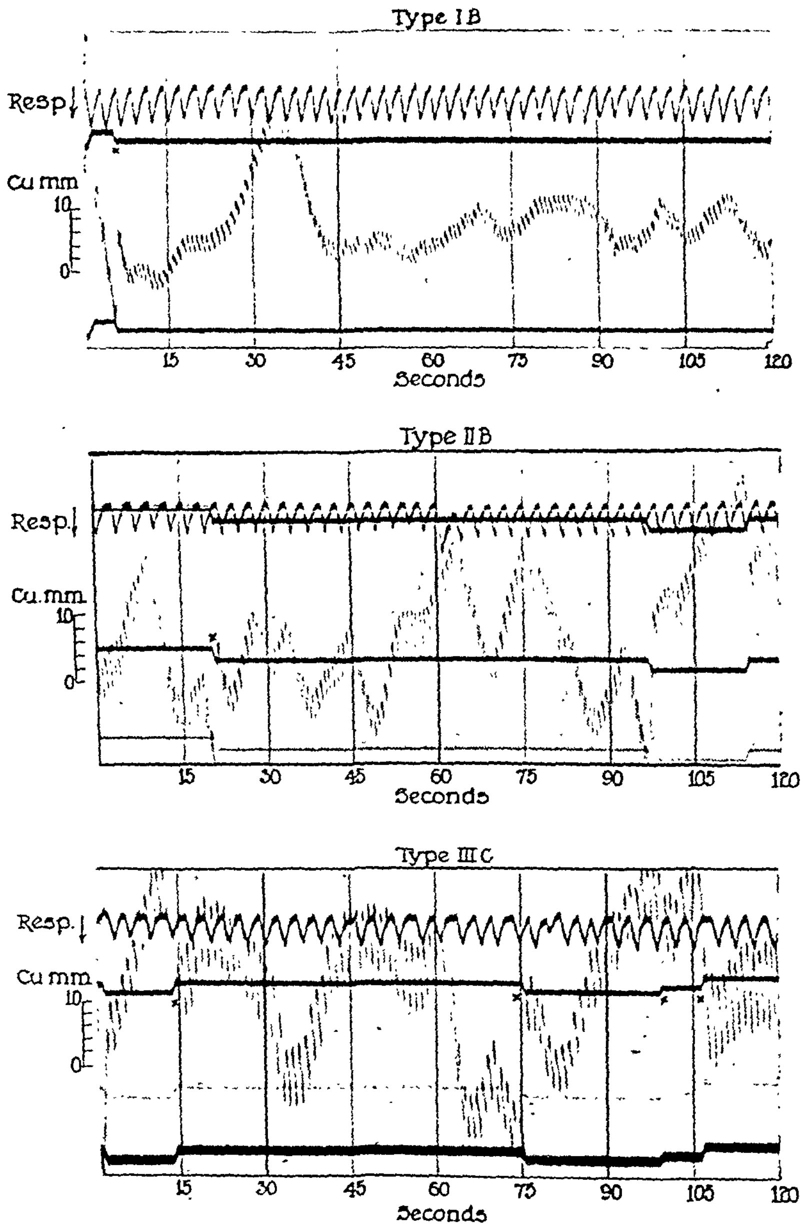

FIG. 1. ORIGINAL RECORDS ARE REPRODUCED TO ILLUSTRATE VARIOUS TYPES OF ALPHA WAVES

In I B, the pulse waves are of uniformly small volume; whereas in III C, there is a considerable variation in volume through the record. The downward direction of the arrow indicates inspiration. Changes in the position of the base-line, to keep the record in the optical field, are indicated by X.

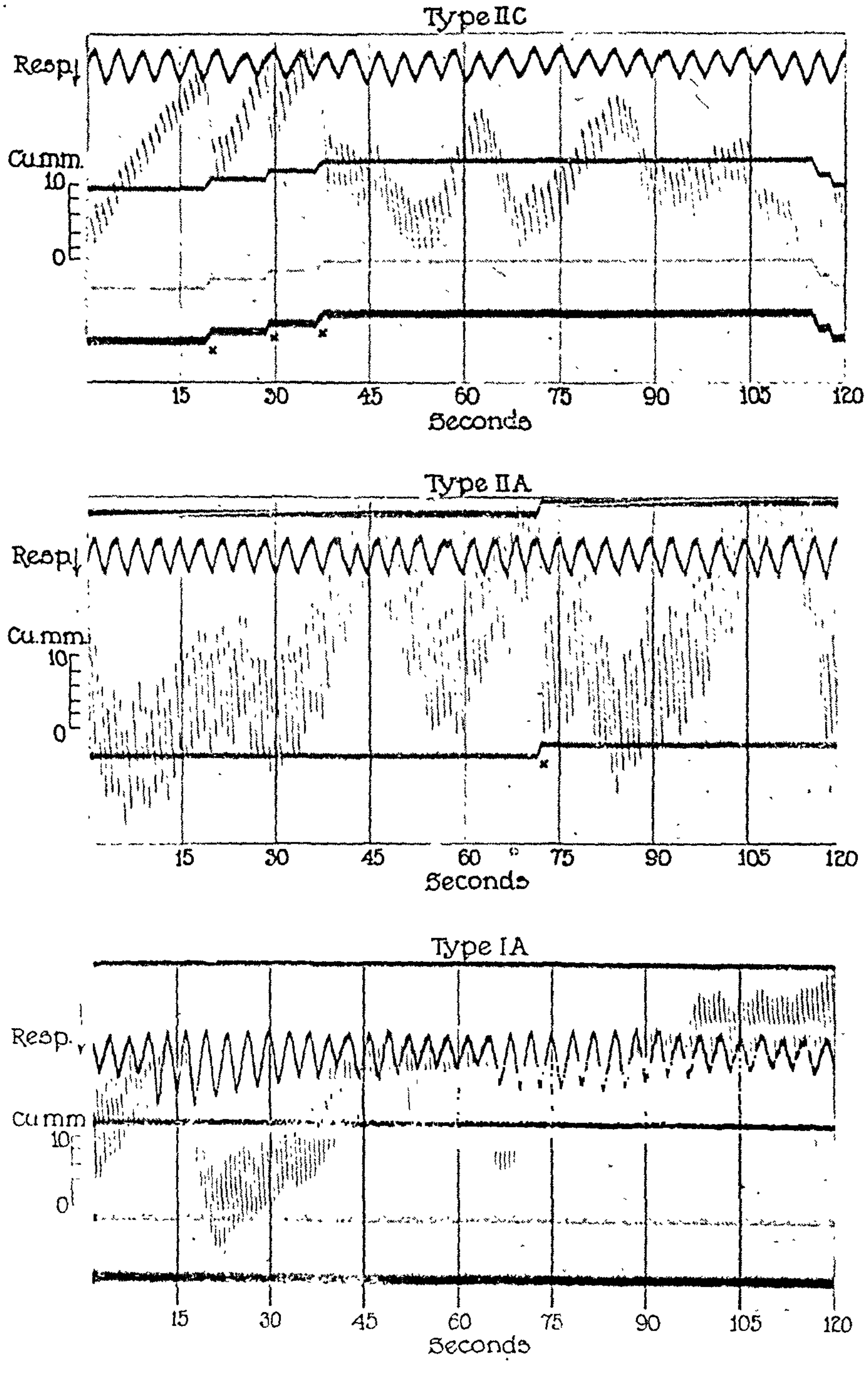

FIG. 2. ORIGINAL RECORDS ARE REPRODUCED TO ILLUSTRATE VARIOUS TYPES OF ALPHA WAVES

The records of Types II C and II A show particularly well how similar alpha waves may be combined with entirely different pulse waves. The downward direction of the arrow indicates inspiration. Changes in the position of the base-line, to keep the record in the optical field, are indicated by X.

were large, others in which they were small, and still others in which small and large ones were found at different times. The decision as to whether the pulse waves were large or small depended upon studying the whole series of records obtained from each individual. To obtain a standard for comparison, small pulse waves (2 to 4 cu. mm.) could always be obtained at the end of each record by asking the subject to inspire deeply. If the pulse waves secured at rest were

of this order of magnitude, they were classed as small; if at least 3 times this magnitude, large (9 to 12 cu. mm.). Large pulse waves were of a size comparable with those obtainable by artificial methods of producing prolonged vasodilatation. Such methods were not employed in the present study, the object having been to obtain records as free as possible from the effects of external influences.

Pulse waves which were predominantly large were called "A," those that were small "B," and those of varying size "C." With 3 types of records of pulse waves and 3 types of records of alpha waves, theoretically 9 combinations are possible. Actually, only 8 were noticed:

I A, with small alpha and large pulse waves;
I B, with small alpha and small pulse waves;
I C, with small alpha and varying pulse waves;
II A, with intermediate alpha and large pulse waves;
II B, with intermediate alpha and small pulse waves;
II C, with intermediate alpha and varying pulse waves;
III A, with large alpha and large pulse waves;
III C, with large alpha and varying pulse waves.

This list permits a choice of 3 types which are clearly distinguishable, both on inspection of the tracing and by measuring. These are I A, I B, and III C (Figures 1 and 2). Although II A, II B, and II C are distinct types, they are intermediate and cannot as readily be identified by inspection alone.

It was apparent that a person's record exhibited, during a given period, consistent characteristics. Just afterwards, he formulated his estimate of his emotional status. The suggestion lay close, as has been said, to making an attempt to explore the possibility of a relation between the two. When the records changed, the question to be answered was, "Was there a change also in his emotional status?" Unfortunately, too few subjects were studied to provide a basis for a statistical study of the results. Definite trends were however detected. When intense anxiety was reported, the plethysmographic records uniformly exhibited small alpha and small pulse waves (Type I B); contentment with its accompanying relaxation went hand in hand with small alpha waves

TABLE I

Comparison of the type of alpha and pulse waves with the dominant emotion

Subject number	Age	Date	Volume of alpha deflections	Average number of alpha deflections	Size of pulse waves	Classification	Dominant emotion	Remarks
	years		*cu. mm.*	*per minute*				
122	29	May 4, 1942	16	4.0	Varying	II C	Resentment, Sadness	Marked resentment present for 3 to 4 days. Cause unknown.
		May 22, 1942	25.7	5.0	Varying	III C	Resentment	
		June 15, 1942	18.6	5.1	Varying	III C	Resentment	
		June 25, 1942	7.6	4.7	Large	I A	Contentment	Duration—several hours.
123	38	April 23, 1942	10	6.0	Large	I A	Contentment	Duration—several hours.
		April 30, 1942	12	4.5	Small	I B	Anxiety	Sudden onset, 2 hours duration, related to necessity of giving a lecture.
		June 5, 1942	18	2.8	Large	III A	Resentment	Slight in intensity, fleeting duration, related to memory of interpersonal situations 5 yrs. previously.
124	27	April 10, 1942	32.1	6.5	Varying	III C	Anxiety	Duration about 7 days, gradual onset, related to subject's imminent marriage (on April 11, 1942).
		May 14, 1942	13.7	6.4	Large	II A	Contentment	Slight anxiety, 8 hours duration, related to sexual topic.
		May 25, 1942	9.7	7.0	Large	I A	Depression	Slight fatigue, slight general muscular tension, mild alcoholic excess previous night.
		June 1, 1942	11.0	5.0	Large	I A	Contentment	Anxiety very slight and fleeting, contentment dominant.
		June 15, 1942	14.3	5.2	Large	II A	Anxiety	Sudden onset just prior to recording, related to telephone call with no opportunity for answering.

TABLE I—*Continued*

Subject number	Age	Date	Volume of alpha deflections	Average number of alpha deflections	Size of pulse waves	Classification	Dominant emotion	Remarks
	years		*cu. mm.*	*per minute*				
125	28	April 21, 1942	10.0	4.5	Varying	I C	Contentment	Duration 6 to 7 hours.
		May 7, 1942	9.0	5.2	Small	I B	Anxiety	Duration 6 hours, sudden onset; related to major operation on father on day of recording and to rejection of subject by an important friend.
		May 12, 1942	19.8	4.0	Varying	III C	Anxiety	Father's illness, situation with his friend improved, emotion less intense than on May 7, 1942.
		May 14, 1942	23	4.3	Varying	III C	Elation	Moderate, duration 4 hours, gradually decreasing intensity related to offer of desirable job and to leaving present position.
		May 27, 1942	17.5	4.4	Varying	II C	Depression	Slight, 3 hours, related to war.
		June 5, 1942	19.9	4.4	Varying	III C	Mixed	Resentment, contentment, anxiety in slight degrees, indefinite durations and causes.
		June 15, 1942	20.6	6.4	Varying	III C	Elation	Moderate intensity, 1 hour duration, related to news of a patient's good progress (others had given poor prognosis).
		June 16, 1942	20.6	5.9	Varying	III C	Anxiety	Duration half hour, moderate intensity, related to complications in father's illness.
		June 18, 1942	18.5	5.9	Varying	III C	Resentment	Slight, duration 2 to 3 hours, related to situation with patient.
126	29	May 1, 1942	14	5.3	Large	II A	Resentment	Slight, related to a delay in the recording.
		June 2, 1942	13.1	5.5	Small	II B	Resentment	Slight, occurred during recording, cause unknown.
127	28	May 11, 1942	16	4.1	Varying	II C	Anxiety	Slight, sudden onset just before recording, cause unknown.
		June 5, 1942	20.2	6.2	Varying	III C	Depression	Slight, duration 7 hours, fatigued, diarrhea and malaise all day.
		June 18, 1942	15.8	6.0	Varying	II C	Anxiety	Slight, sudden onset during recording, related to thoughts of work.
128	30	May 29, 1942	19.5	5.3	Varying	III C	Elation	8 hours duration, moderate intensity, related to friend getting a job.
		June 19, 1942	23	4.8	Varying	III C	Resentment	Onset during test, related to his need to use time otherwise.
129	30	May 6, 1942	21.6	6.2	Varying	III C	Anxiety	Moderate intensity 10 days, gradual onset, related to approaching marriage.
		June 2, 1942	18.3	6.6	Varying	III C	Anxiety	As above.
		June 25, 1942	44.3	5.4	Varying	III C	Elation Resentment	Post marital (2 weeks) elation, moderate degree; resentment slight, with onset during recording, related to wish to be doing something else.
130	32	May 18, 1942	15.3	4.2	Varying	II C	Contentment Anxiety	Slight degrees of both, both present 5 to 10 days, causes unknown.
131	28	May 19, 1942	16.3	8.0	Small	II B	Contentment Anxiety	Both slight. Anxiety present 2 hours and related to preparation of lecture material.
		May 23, 1942	14.3	7.8	Small	II B	Anxiety	Moderate intensity, duration 4 days, related to above.
		May 26, 1942	13.0	8.3	Small	II B	Resentment Anxiety	"Resented being experimented upon," moderate intensity. Anxiety as above—lectures to start June 1, 1942.
		June 1, 1942	14.4	7.4	Varying	II C	Anxiety Elation	4 hours after first lecture; anxiety less, slight elation.
		June 9, 1942	25.3	4.8	Varying	III C	Anxiety	Slight, 6 hours, related to sexual topics.
		June 19, 1942	12.0	7.1	Large	I A	Contentment	Gradual onset, 8 hours duration.

TABLE II

Comparison of the intensity of certain emotions with type of alpha wave at time of recording

Emotion	Subject number	Date	Volume of alpha deflections	Volume of pulse waves	Average number of alpha deflections	Classification of alpha wave record	Intensity of emotion
			cu. mm.	*cu. mm.*	*per minute*		
Anxiety	127	May 11, 1942	16.0	Varying	4.1	II C	+
	127	June 18, 1942	15.8	Varying	6.0	II C	+
	129	May 6, 1942	21.6	Varying	6.2	III C	++
	131	June 9, 1942	25.3	Varying	4.8	III C	++
	124	June 15, 1942	14.3	Large	5.2	II A	++
	124	April 10, 1942	32.1	Varying	6.5	III C	+++
	125	May 12, 1942	19.8	Varying	4.0	III C	+++
	129	June 2, 1942	18.3	Varying	6.6	III C	+++
	131	May 23, 1942	14.3	Varying	7.8	II B	+++
	125	June 16, 1942	20.6	Varying	5.9	III C	++++
	125	May 7, 1942	9.0	Small	5.2	I B	++++
	123	April 30, 1942	12.0	Small	4.5	I B	++++
Resentment	123	June 5, 1942	18.0	Large	2.8	III A	+
	126	May 1, 1942	14.0	Large	5.3	II A	+
	126	June 2, 1942	13.1	Small	5.5	II B	+
	128	June 19, 1942	23.0	Varying	4.8	III C	++
	125	June 18, 1942	18.5	Varying	5.9	III C	++
	122	June 15, 1942	18.6	Varying	5.1	III C	++
	122	May 22, 1942	25.7	Varying	5.0	III C	++
Elation	125	May 14, 1942	23.0	Varying	4.3	III C	++
	125	June 15, 1942	20.6	Varying	6.4	III C	++
	128	May 29, 1942	19.5	Varying	5.3	III C	++
Depression	125	May 27, 1942	17.5	Varying	4.4	II C	+
	124	May 25, 1942	11.0	Large	5.0	I A	+
	127	June 5, 1942	20.2	Varying	6.2	III C	++
Contentment	122	June 25, 1942	7.6	Large	4.7	I A	++
	124	June 1, 1942	11.0	Large	5.0	I A	++
	125	April 21, 1942	10.0	Varying	4.5	I C	++
	131	June 19, 1942	12.0	Large	7.1	I A	+++
	123	April 23, 1942	10.0	Large	6.0	I A	+++

and large pulse waves (Type I A). There were 2 variants; one with intermediate alpha and large pulse waves (Type II A) and one with small alpha and varying pulse waves (Type I C). The former was borderline, the alpha waves being only 0.7 cu. mm. larger than the usual I A alpha waves; the other, also borderline, exhibited varying instead of uniformly large pulse waves. Anxiety of moderate degree, elation, and resentment were commonly associated with records having large alpha waves and varying pulse waves (Type III C) (Tables I and II).

These 3 groups were distinct. Plethysmographic records of the intermediate types (II A, II B, II C, III A) were associated with reports of slight resentment or slight anxiety, sadness, or a combination of various emotions, none of which was dominant. No uniformity was present in the records of the subjects reporting depression. Of these 3 records, one was Type II C, another III C, and the third, I A. At no time was a change in the type of the alpha wave-pulse wave configuration noticed in any of the groups during the course of a single observation, nor was a change in emotional status.

In 2 subjects, records were made during sleep, at short intervals throughout an entire night. Sleep was continuous and not restless. Alpha deflections occurred throughout the night; unfortunately, neither subject remembered any dreams so that a comparison of the records with their emotional state was impossible. In point of fact, records obtained during sleep resembled those obtained just before and after. Their emotional status before and after likewise remained unchanged. They were in a state of moderate anxiety.

DISCUSSION

Comparison of plethysmographic records with reports of concomitant emotional states has pro-

lpha waves with varying size of pulse waves Type III C).

Individual subjects at rest, free from recog-iizable external stimuli and not required to carry on intellectual activity, have exhibited changing ecords. This kind of variability has been found o go closely hand in hand with changes in emo-ional status. Certain records (Type I A) were obtained only from subjects in a state fully re-axed and contented. Certain others (Type I B) occurred when anxiety was dominant. With less anxiety or with elation or resentment, Type III C records were obtained. With depression, no uniform alpha-pulse wave pattern was observed. Slight resentment or slight anxiety or a combination of various emotions, none of which was dominant, occurred with intermediate types of records. On the assumption that changes in the degree of activity of the autonomic nervous system parallel changes in emotional status, an explanation is proposed for the changes based on changes in the autonomic nervous system, the results of which have been observed in alpha and in pulse waves and in combinations of the two.

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SOME EFFECTS OF LOW ATMOSPHERIC PRESSURE ON RATS [1]

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This paper reports results obtained on rats subjected to prolonged exposure to low atmospheric pressure. Histological studies reveal depletion of adrenocortical lipid during the early phase of exposure. Chemical analyses are presented of the muscle and liver in rats exposed to low atmospheric pressure while on a normal diet, after a diet low in potassium, and after receiving desoxycorticosterone acetate. Deficit of body potassium—the typical change in muscle produced by some of the cortical steroids—decreases the resistance to low atmospheric pressure. Furthermore, low atmospheric pressure aggravates the tendency of desoxycorticosterone acetate to produce cardiac lesions. Some features of the muscle analyses suggest overactivity of the adrenal cortex during exposure to low atmospheric pressures.

METHODS

Only male albino rats of the Wistar strain, weighing from 200 to 250 grams, were used. The basic feed was Purina Fox Chow which contains 16 mM of K per 100 grams. The diet low in potassium contained lactalbumin, sucrose, Crisco, yeast, and a salt mixture and has been previously described (1). It contained 1.6 mM per 100 grams. Desoxycorticosterone acetate (DOCA) was injected subcutaneously in doses of 2 mgm. daily for 14 days before exposure to low atmospheric pressures and also during exposure. For this purpose, crystalline desoxycorticosterone acetate was dissolved in hot alcohol and precipitated in a fine suspension which contained 2 mgm. of the compound per cc. in 5 per cent alcoholic physiological saline.

The rats were exposed to low barometric pressures in bell jars with valves which maintained a given reduced pressure within fairly narrow limits and permitted adequate renewal of air, so that the effect was essentially reduction of atmospheric pressure.[2] The animals were kept continuously at the reduced pressure, except for the hour daily needed for cleaning the cages and replacing food and water. Only two pressures were used: one equivalent to an elevation of 20,000 feet above sea level and the other equivalent to 25,000 feet.

About one-half hour after being removed from the bell jars, the rats were first anesthetized with nembutal, then one gastrocnemius and a piece of liver were removed for determination of glycogen, and finally, the rats were bled to death by withdrawing blood from the abdominal aorta. Blood, muscle, and liver were analysed chemically as in previous studies (2). The adrenals were placed in 4 per cent solution of formaldehyde for 2 days and weighed to the nearest milligram after removal of adventitia. Paraffin sections were stained with hematoxyline and eosin; frozen sections (10 to 15 microns thick) were stained with Sudan IV. The phenylhydrazine and digitonin techniques of Bennett (3) were used in selected cases to demonstrate corticosteroids and cholesterol, respectively. Since, in rats, the distribution of lipid (Sudan IV staining) is essentially the same as the distribution of cholesterol (digitonin technique) or corticosteroids (phenylhydrazine technique), the simple Sudan IV staining serves to indicate distribution of all fractions of cortical lipids.

RESULTS

The interpretation of the results is complicated by the fact that exposure to low atmospheric pressure leads to varying degrees of starvation and loss of weight and, for this reason, fasting controls are necessary. At 20,000 feet, the rats were distressed during the first 2 to 3 days but gradually became acclimatized so that they appeared fairly well at the end of the week. No food was eaten during the first day but the appetite gradually returned, so that approximately normal amounts were taken after the third day. In this respect, the rats receiving DOCA were different from those of the other groups, for they remained distressed and never recovered their appetite, even at 20,000 feet. One rat receiving DOCA died on the third day at 20,000 feet while none of the others died at this level. All rats remained extremely distressed at 25,000 feet and practically no food was taken although water was drunk. No rat receiving DOCA was able to sur-

[1] Aided by a grant from the Fluid Research Fund of the Yale University School of Medicine.

[2] The decompression chambers were kindly placed at our disposal by the Department of Physiology. Determination of the rate of ventilation showed that significant accumulation of carbon dioxide would not develop during these experiments.

vive 24 hours at 25,000 feet. Table I shows the weight losses expressed in per cent of initial body weight. These values may be considered measures of the spontaneous fasting as well as the degree of distress during the exposure to low atmospheric pressures.

Adrenal weight

The data on the weights of the adrenals are summarized in Table I. Since adrenal weight varies directly with body weight (4, 5) and since adrenals show minimal changes in size with transient fluctuations in body weight (5), the adrenal weights are expressed per unit of initial body weight. During the first 2 days at 25,000 feet, the adrenals are statistically enlarged in the rats fed the diet low in potassium but are normal in this group at the end of a week at this pressure. One normal rat exposed for 7 days at 25,000 feet shows absolute enlargement but this rat is apparently an exception since he showed considerably more distress and loss of weight than the other rats of this group. Otherwise, the variations in adrenal size are within the range of the fasted controls. While the number of observations is small, the data are sufficient to show that great

TABLE I

Adrenal size and cortical lipid

	Number of rats	Period of exposure	Weight loss	Ratio of adrenal to body weight: Initial weight	Ratio of adrenal to body weight: Final weight	Cortical lipid
			per cent	*grams per kgm.*		
Rats kept at sea level						
Normal diet	8			0.145±0.005		++++
Low K diet	10			0.133±0.003		++++
DOCA 14 days	5			0.131±0.005		++++
Fasted 1 day	3		12	0.151±0.005	0.172	++++
Fasted 2 days	3		15	0.164±0.004	0.194	++++
Fasted 7 days	3		28	0.158±0.004	0.224	++
Rats exposed to low atmospheric pressures						
Normal rats 20,000 feet	3	1 day	9	0.155±0.013	0.171	+++
	3	2 days	11	0.154±0.008	0.172	++
	3	7 days	11	0.131±0.013	0.142	++++
Normal rats 25,000 feet	2	1 hour	2	0.128±0.003	0.131	++++
	2	2 hours	3	0.152±0.010	0.157	+++
	2	5 hours	3	0.141±0.010	0.148	++
	3	1 day	13	0.136±0.002	0.155	+
	3	2 days	18	0.158±0.002	0.192	0
	3	7 days	24	0.151±0.004	0.198	++++
	1*	7 days	37	0.204	0.325	0
Low K diet 20,000 feet	4	1 day	10	0.143±0.013	0.159	++
	2	2 days	7	0.131±0.014	0.142	++
	2	7 days	10	0.115±0.003	0.128	++++
Low K diet 25,000 feet	3	1 day	10	0.196±0.012	0.216	++
	3	2 days	9	0.204±0.010	0.224	+
	3	7 days	16	0.159±0.009	0.189	++++
DOCA 20,000 feet	3	1 day	8	0.146±0.011	0.159	++
	3	2 days	13	0.157±0.066	0.208	0
	3	7 days	16	0.135±0.007	0.150	++++
DOCA 25,000 feet	6**	1 day	8	0.147±0.005		++
KCl in water 25,000 feet	2	6 days	28	0.136±0.014	0.190	++++

Cortical lipid: ++++, normal; +++, slight reduction; ++, moderate reduction; +, extensive reduction; 0, apparent absence.

* Absolute hypertrophy and lipid depletion in rat exhibiting extreme loss of weight.

** 5 of 6 rats died in less than 12 hours.

changes in adrenal size do not regularly accompany prolonged exposure to pressures equivalent to 20,000 or 25,000 feet during the first 7 days.

Adrenocortical lipids

The histologic appearance of the adrenals is represented in Table I in 5 degrees: ++++ indicating the normal appearance of the Sudan IV stain (see Figure 1, Part 1); +++, ++, and +, stages in the partial depletion of cortical lipids (see Figure 1, Parts 2, 3, and 4); 0, apparently complete loss of cortical lipid. Histologically, the adrenals present marked evidence of loss of lipid during the early period of exposure but with subsequent recovery. At 20,000 feet, the loss after 1 or 2 days is definite in normal rats, somewhat greater in rats on the diet low in potassium, and very marked in the rats receiving DOCA. On the second day, the adrenals of rats receiving DOCA show practically no sudanophilic staining. In this group, the loss of cortical lipid is in addition to the depletion in a narrow peripheral zone of the glomerulosa which has been described following injections of the synthetic cortical compound (6). After an exposure of 7 days, all animals able to withstand either pressure show normal lipid patterns in the adrenals. This finding is especially remarkable because, at 25,000 feet, rats are apparently unable to become fully acclimated and would be unable to survive much longer than a week at this pressure.

Studies on normal rats show that the losses of cortical lipid start very early. Although no significant changes are noted at 25,000 feet after 1 hour, the beginnings of focal lipid alterations are quite definite after 2 hours, the sudanophilic material being in smaller droplets as well as less widely distributed (Figure 1, Part 2); after 5 hours, depletion of cortical lipid is even more striking (Figure 1, Part 3). Partial recovery is revealed after 3 days' exposure (Figure 1, Part 6) and is complete after 7 days (Figure 1, Part 7). The use of the digitonin and phenylhydrazine techniques demonstrates that changes in cortical cholesterol and corticosterones parallel the changes in lipid, as revealed by staining with Sudan IV. Thus, the loss of lipid probably starts immediately, attains a maximum in 1 or 2 days when recovery commences, and becomes complete within a week.

Liver and kidney

Microscopic examinations of the liver and kidneys were made in only a few of the animals in each group. Many of the livers in each group showed extensive vacuolization of parenchymal cells, associated with sudanophilic material in the central portion of the liver lobule, but the typical picture of chronic passive congestion was not encountered. No changes were noted in the kidneys except the relative increase in size when compared to final body weight. This finding was to be expected, since kidneys in acute inanition retain their size almost as well as the adrenals. As has been previously pointed out (7), diets low in potassium or treatment with DOCA lead to enlargement of the kidneys. The kidneys were relatively large in rats on the diet low in potassium and in those receiving DOCA but diminished oxygen tension did not alter this state.

Heart

Histological sections of practically all hearts were studied. No lesions were encountered except in rats receiving desoxycorticosterone acetate. Table II brings together the data which indicate that low oxygen tension aggravates the tendency to lesions produced by injections of DOCA. As was previously pointed out (8), lesions can be found in over two-thirds of the rats kept at sea level while receiving DOCA for 30 days, but microscopic lesions are quite rare at sea level when injections are continued for only 14 days, even if a period of fasting is superimposed thereafter. However, lesions were regularly found—some ex-

TABLE II

Cardiac lesions after DOCA

Diet	Injections	Fast	Low O_2	Number of rats	Number of lesions
	days	*days*	*days*		
N	30	0	0	50	34
N	14	0	0	7	1
N	14	1 to 7	0	8	0
N	14	0	1 to 7	8	8
LK	14	0	3	4	0

When fasting or exposure to low oxygen tension was carried out, injections of DOCA (2 mgm. daily) were continued in addition to the preparatory 14 days. N and LK refer to normal and low potassium diets, respectively. Exposures were equivalent to an elevation of 20,000 feet

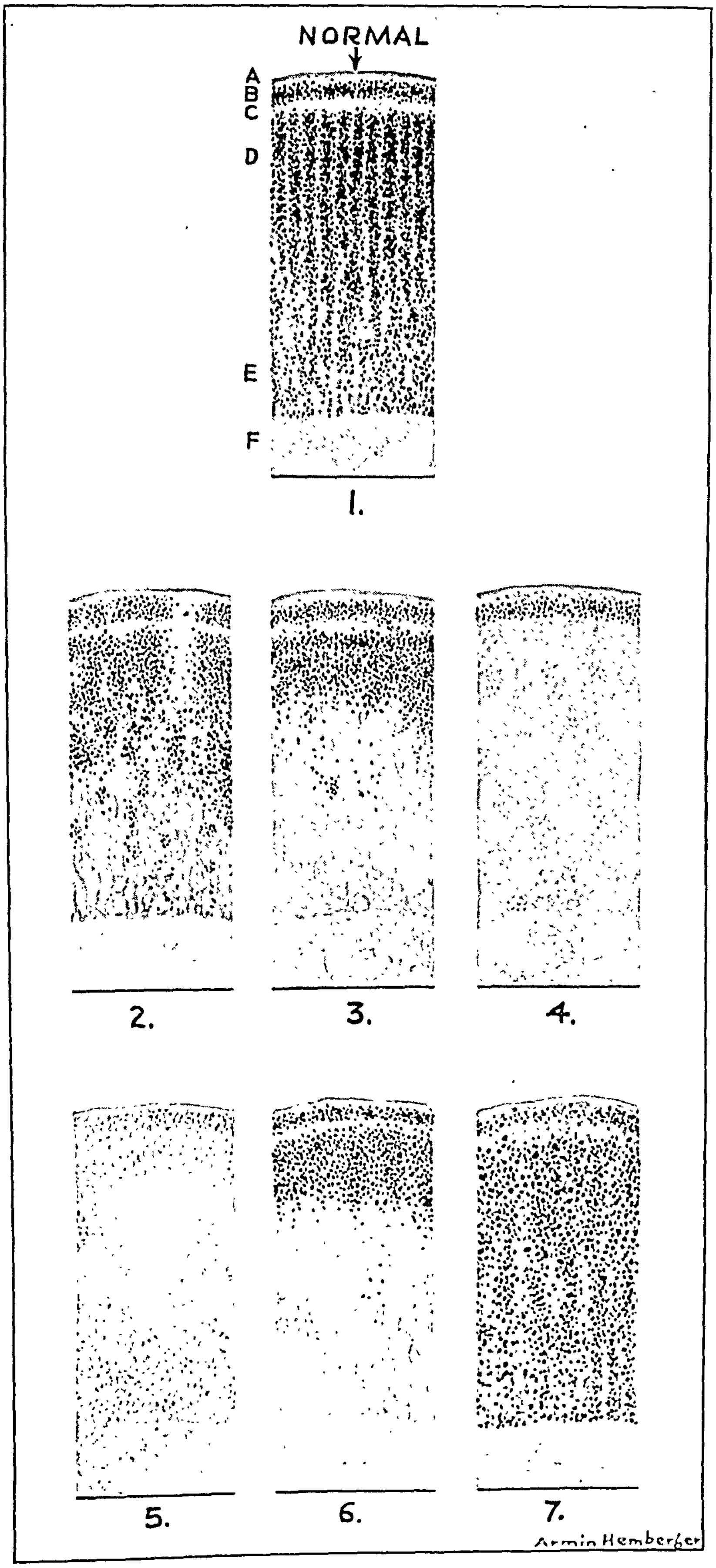

Fig. 1

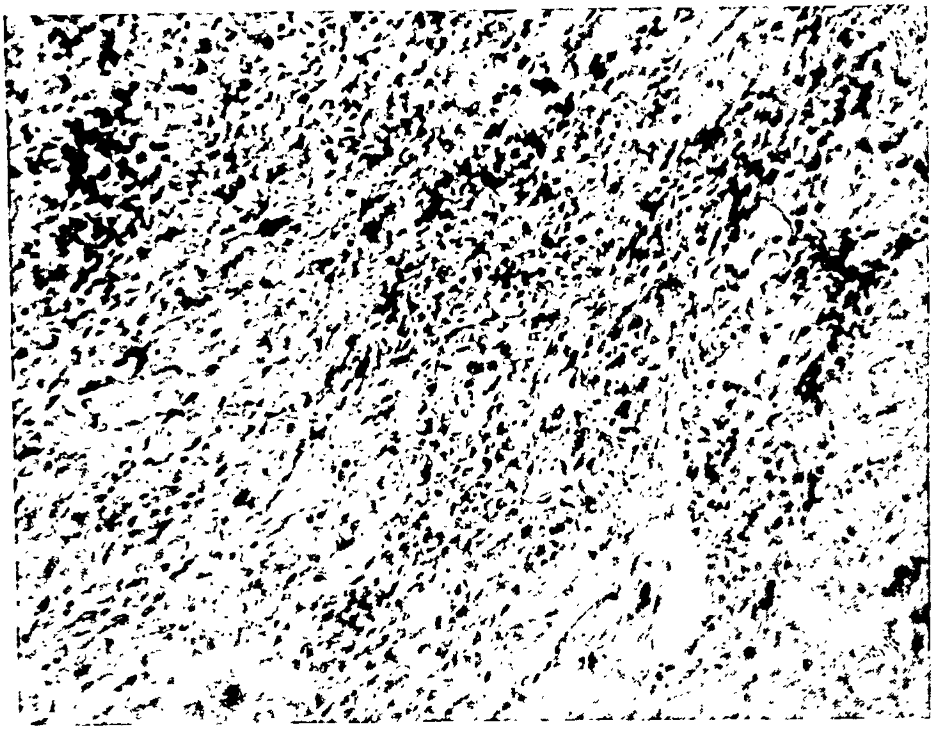

FIG. 2. RAT 31. PHOTOMICROGRAPH OF THE HEART

This rat received 2 mgm. of desoxycorticosterone acetate subcutaneously each day for 14 days; he was exposed to an atmospheric pressure equivalent to an elevation of 20,000 feet for 1 day.

tremely extensive as is shown in the photomicrograph (Figure 2)—when exposure to low oxygen tension was carried out for 1 to 7 days after injections of DOCA. Since lesions were not found when rats had received a diet low in potassium before exposure to low oxygen tensions, depletion of body potassium before exposure is not the sole cause of these lesions. This conclusion follows from the fact that roughly comparable losses of body potassium were produced both by a diet low in potassium and by repeated injections of DOCA. Low oxygen tensions or deficit of carbon dioxide apparently aggravate the tendency to cardiac lesions produced by repeated injections of DOCA.

FIG. 1. DIAGRAMMATIC DRAWINGS OF SECTIONS FROM FROZEN ADRENALS STAINED WITH SUDAN IV. MAGNIFIED ABOUT 25-FOLD

Part 1. Normal rat adrenal. The black dots represent sudanophilic material. A. Thin, acellular fat-free capsule; B. glomerulosa layer; C. "clear zone" of outer fasciculata; D. outer fasciculata; E. inner fasciculata; F. medulla.

Part 2. Exposure: 25,000 feet for 2 hours. Note the slight reduction of the cortical lipid.

Part 3. Exposure: 25,000 feet for 5 hours. Note the moderate reduction of cortical lipid.

Part 4. Exposure: 25,000 feet for 1 day. Note the complete absence of cortical lipid save for that present in the glomerulosa layer.

Part 5. Exposure: 25,000 feet for 2 days. Note the complete absence of cortical lipid.

Part 6. Exposure: 25,000 feet for 3 days. Note the beginning of return of cortical lipid in the glomerulosa and outer fasciculata.

Part 7. Exposure: 25,000 feet for 7 days. Note the relatively normal lipid pattern of the adrenal cortex.

CHEMICAL CHANGES IN SERUM AND TISSUES

The chemical composition of serum, muscle, and liver was determined because of the important changes in serum known to take place and because these changes might be accompanied by alterations in the tissues. Furthermore, the suggestive evidence of overactivity of the adrenal cortex brings up the question of changes characteristic of overactivity or deficiency of these glands.

The muscle and serum analyses are given in Tables III, IV, and V. Note that sodium and chloride of the serum are expressed per liter of ultrafiltrate, while water and potassium are expressed per liter of serum. In the case of Na and Cl, the concentrations are calculated using the water content and an average Donnan factor of 0.96. These concentrations represent approximately those in extracellular fluid. A similar calculation was not made in the case of potassium since the distribution of this ion is uncertain. Two types of derived data are given in the last 2 columns. The first may be called the extracellular water and was calculated by the following formula:

$$(H_2O)_e = \frac{(Cl)_t - 1}{[Cl]_e} \times 1000$$

in which $(H_2O)_e$ is extracellular water in ml.; $(Cl)_t$, total tissue chloride in mM; $[Cl]_e$, the concentration of chloride in mM per liter of an ultrafiltrate of serum. The reason for subtracting 1 is that it represents approximately the chloride that does not behave like extracellular chloride (9, 10). The other derived datum may be called

TABLE III

Serum and muscle of rats fed normal diet

Number of rats	Days*	Elevation	Per L of serum		Per L of serum ultrafiltrate		Per 100 grams of fat-free muscle solids						$(H_2O)_e$	$(Na)_i$
			H_2O	K	[Cl]	[Na]	H_2O	Cl	Na	K	P	N		
		feet × 10^3	*grams*	*mM*	*mM*	*mM*	*grams*	*mM*	*mM*	*mM*	*mM*	*grams*	*ml.*	*mM*
13	0	0	925	4.0	113	147	340	6.1	9.7	48.7	32.4	15.3	45	3.1
			±6	±0.8	±3.3	±3	±6	±0.6	±0.8	±1.1	±1.5	±0.2		
11	1	0	941	4.6	112	144	344	6.5	9.2	47.2	33.6	15.4	49	2.1
			±5	±0.5	±4	±3	±8	±0.8	±0.4	±1.0	±0.8	±0.2		
4	7	0	951	4.4	113	143	353	8.5	11.7	44.0	33.0	15.2	66	2.2
6	1,2	20	929	4.6	124	144	343	6.6	9.4	48.2	35.5	15.5	45	0.1
3	7	20	931	5.6	124	153	349	8.1	14.5	48.2	34.6	15.6	57	5.8
6	1,2	25	931	4.9	127	148	325	6.7	9.0	49.3	34.4	15.2	45	2.4
3	7	25	945	5.6	138	145	342	9.1	12.0	45.0	32.5	15.1	59	3.5
2	7**	25	932	5.2	120	149	347	8.5	10.2	49.3	33.0	15.1	62	0.9

* Days refers to duration of fast in the case of the first 3 groups and duration of the exposure to low atmospheric pressures in the remaining groups. Standard deviations are given for the controls but not for the others. The figures for the experimental group represent average values.

** Rats exposed to 25,000 feet for 7 days while receiving water containing 0.5 per cent KCl to drink.

TABLE IV

Serum and muscle of rats injected with DOCA

Number of rats	Days*	Elevation	Per L of serum		Per L of serum ultrafiltrate		Per 100 grams of fat-free muscle solids						$(H_2O)_e$	$(Na)_i$
			H_2O	K	[Cl]	[Na]	H_2O	Cl	Na	K	P	N		
		feet × 10^3	*grams*	*mM*	*mM*	*mM*	*grams*	*mM*	*mM*	*mM*	*mM*	*grams*	*ml.*	*mM*
21	0	0	941	3.4	110	150	328	6.3	14.4	40.0	32.0	15.4	48	7.2
			±6	±0.8	±3	±6	±9	±0.7	±1.5	±2.4	±1.1	±0.1		
4	1	0	940	4.4	100	141	340	6.1	12.8	40.0	33.5	15.2	51	5.6
2	7	0	948	4.7	100	149	343	7.3	14.6	40.0	31	14.8	63	5.2
6	1,2	20	940	3.4	119	149	348	9.2	15.0	42.0	34	15.6	69	8.1
3	7	20	943	4.5	113	143	346	7.3	17.7	39.0	33	15.2	54	10.0

* Days refers to the duration of the fast or exposure to low atmospheric pressures. The first 3 groups are the fed and fasted controls. All rats received DOCA for 14 days before as well as during the fast or exposure to low tensions of oxygen.

TABLE V

Serum and muscle of rats receiving low K diet

Number of rats	Days*	Elevation	Per L of serum		Per L of serum ultrafiltrate		Per 100 grams of fat-free muscle solids						$(H_2O)_e$	$(Na)_i$
			H_2O	K	[Cl]	[Na]	H_2O	Cl	Na	K	P	N		
		feet × 10^3	*grams*	*mM*	*mM*	*mM*	*grams*	*mM*	*mM*	*mM*	*mM*	*grams*	*ml.*	*mM*
15	0		941	2.5	106	146	333	6.6	15.1	39.6	32.0	15.1	53	7.4
			±1.2	±0.3	±4	±3	±6	±0.7	±1.0	±2.1	±0.9	±0.2		
6	1,2	20	930	4.5	121	151	312	6.8	11.3	43.0	33.0	15.2	56	2.8
3	7	20	946	4.5	130	147	339	8.9	13.1	44.3	33.0	15.3	60	4.3
6	1,2	25	936	3.3	121	148	318	5.9	10.6	44.0	35.0	15.1	40	4.6
3	7	25	936	4.4	124	150	323	8.5	13.1	41.0	32.0	15.1	60	4.0

* Days refers to the duration of the fast or exposure to low atmospheric pressures. The first group represents fed controls. All rats received the diet low in potassium for 14 days before the exposure.

intracellular sodium. It is:

$$(Na)_i = (Na)_t - \frac{[Na]_e(H_2O)_e}{1000}$$

where $(Na)_i$ is intracellular sodium; $[Na]_e$, the concentration in serum ultrafiltrate; and $(H_2O)_e$, the extracellular water. At each elevation, 3 animals were exposed for 1, 2, and 7 days. When there are no statistically different values on the first and second days, these results are combined. Fasting controls are given for the normal and DOCA groups.

Serum analyses

The data suggest that rats develop alkalosis in the serum during exposure to low atmospheric pressures. In the normal rats (Table III), the concentrations of chloride in serum ultrafiltrate rise from 113 to 120, or more, mM per liter and this change is not accompanied by a significant change in serum sodium. Indirectly, these findings indicate that the concentration of bicarbonate in serum decreases. Not shown in the tables are analyses of 6 normal rats, exposed for 1, 2, and 4 hours at 25,000 feet. Since these serums showed no changes, the alterations in serum sodium and chloride require between 4 and 24 hours' exposure to low atmospheric pressures before they are manifest. As may be seen from Tables III, IV, and V, normal rats at sea level show higher serum chlorides than rats fed a diet low in potassium or those injected with DOCA. On fasting, rats receiving DOCA develop still lower concentrations of chloride in serum. However, on exposure to low atmospheric pressures, serum chloride increases practically as much in the rats on the diet low in potassium, or in those receiving DOCA, as in the normal rats similarly exposed. These findings at low atmospheric pressures represent the long recognised compensatory reactions accompanying deficit of carbon dioxide which accompany the overbreathing of anoxemia (11). The data show that neither deficit of body potassium nor injection of desoxycorticosterone acetate interferes with this reaction.

Muscle analyses

The muscles show certain changes in water content which are not primarily dependent on exposure to low atmospheric pressure. Thus, normal rats show an increase in extracellular water after a fast of 7 days and after an exposure for 7 days to low atmospheric pressure (see Table III). Rats receiving DOCA show an increase in extracellular water of muscle only during the first 2 days' exposure to low atmospheric pressure (see Table IV). The rats on the diet low in potassium show only questionable increase in extracellular water after 7 days' exposure at 20,000 or 25,000 feet elevation. Since similar changes can be produced in a few minutes by exercise (12), they are not regarded as a fundamental alteration in muscle composition. Within the fibers, certain muscles show changes which are manifested by alterations in the usual relationships between the concentrations of sodium, chloride, and potassium to each other and to the fat-free solids. In normal rats, muscle potassium varies between 47 and 49 mM per 100 grams of fat-free solids but may decrease to 44 mM without the appearance of low

concentration of *potassium* in serum or significant increase in intracellular sodium (1). The data show this type of decrease in muscle potassium without increase in intracellular sodium in (*1*) normal rats fasted for 7 days and (*2*) normal rats after an exposure for 7 days at 25,000 feet. In the case of normal rats exposed to 25,000 feet for 7 days, the administration of 0.5 per cent potassium chloride in the drinking water prevents this loss of potassium but does not alter the evidences of distress at this low atmospheric pressure. Since normal rats at 25,000 feet refuse all food, loss of muscle potassium without increase in intracellular sodium is regarded as a result of fasting rather than anoxemia.

On the other hand, other experiments give evidence of increase in intracellular sodium which is apparently dependent on exposure to low atmospheric pressures. In the normal rats, the value for $(Na)_i$ is distinctly high after 7 days at 20,000 (see Table III). The increase in intracellular sodium occurs early as well as late in the rats treated with DOCA and is very marked after 7 days (see Table IV). Attention is directed to the facts that the rise in $(Na)_i$ is superimposed on an already high value in the DOCA rats and that no statistically significant fall in the already low value for potassium is found. Since similar rises in intracellular sodium are not found in the fasted DOCA rats at sea level, this finding is associated with anoxemia rather than loss of appetite.

As illustrated in Table V, similar increase in intracellular sodium is only suggested in the rats on the diet low in potassium. In the first place, $(Na)_i$ starts at a high value and exposure to low atmospheric pressure leads to a decrease which is accompanied by an increase in muscle potassium. Since the only food offered was the diet low in potassium and this was taken poorly during the first days at 20,000 feet and refused almost entirely at 25,000 feet, the rise in muscle potassium *represents retention* of body potassium freed by consumption of tissues. The rise in $(Na)_i$ from the low value during the first days at 20,000 feet to a higher value after 7 days probably represents the same phenomenon as was observed in normal rats or those receiving DOCA. Attention is directed to the fact that initially the composition of the muscle is about the same in the rats given the diet low in potassium and in those receiving DOCA. Since the rise in $(Na)_i$ is greater in the DOCA rats, the initial composition of the muscle is not the cause of the size of the change. Furthermore, an increase did not develop with fasting alone in the DOCA rats. One may conclude, therefore, that injections of DOCA aggravate this accumulation of intracellular sodium which may be produced in normal rats by anoxemia.

Liver analyses

The liver analyses were undertaken because of the apparent vulnerability of the liver to anoxia and disturbances in circulation, such as shock and cardiac failure. The analyses for water and electrolyte were carried out on pooled samples of 3 livers after aliquots had been removed for determination of glycogen. The values for the latter are given as averages calculated per 100 grams of fat-free solids.

Liver weight

The data show distinct enlargement of the liver accompanying exposure to low atmospheric pressures. The finding is complicated by the fact that

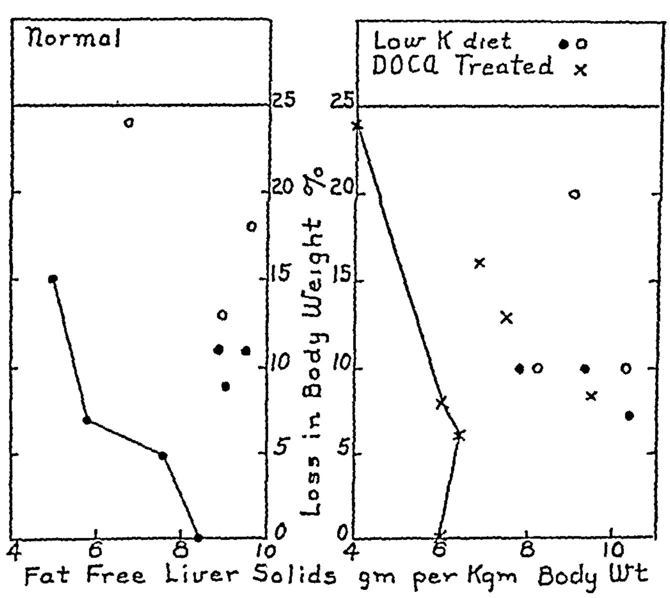

FIG. 3. RELATION OF FAT-FREE LIVER SOLIDS TO LOSSES OF BODY WEIGHT

The points connected by lines represent data from fasted rats while the other points represent data from rats exposed to low atmospheric pressures. The dots and circles represent exposures to simulated elevations of 20,000 and 25,000 feet, respectively. The crosses represent DOCA treated rats exposed to 20,000 feet.

fasting reduces liver size and in some experiments the two effects cancel one another.

In Figure 3, the fat-free solids in per cent of initial body weight are charted against loss in body weight expressed in grams per kilogram of initial weight. One part of the figure presents data on rats normal at the beginning of the experiment, while the other part gives data on the two types of rats depleted of body potassium. The points connected with lines represent data from fasted controls while the other points represent data from rats exposed to low atmospheric pressure.

The chart shows that the losses of body weight at low atmospheric pressure are not accompanied by decreases in liver substance except in the normal rats exposed for 7 days to 25,000 feet. In this case, the refusal of food leads to the same decrease in liver size as is found in the fasting rats. The other rats at reduced pressures show normal or enlarged livers. The size is greatest in rats on the diet low in potassium. The enlargement is not statistically significant unless comparison is made with the fasted rather than the fed controls. The same evidence of liver enlargement is found when water and fat are taken into account in comparing the various livers.

Liver composition

Table VI gives the composition of the livers. In fasting normal rats, the striking changes are the increase in liver fat on the first and second days and increase in liver water after 7 days. The partition of liver water cannot be calculated with certainty.[3] In rats treated with DOCA, the effects of fasting are similar but with this difference. The fed controls receiving DOCA have high Cl, Na, and water, indicating high extracellular water. Fasting reduces the excessive extracellular water. Furthermore, after 1 week of fasting, the livers show a reduced concentration of potassium.

[3] Usually, it is unjustifiable to calculate the value of extracellular water from the liver chloride since the liver chloride is higher than the sodium, indicating considerable intracellular chloride. Neither is it justifiable to calculate extracellular water from liver sodium since there is considerable evidence of the presence of intracellular sodium (9). For these reasons, one cannot assign the changes in liver water to either of the phases of liver water with certainty.

TABLE VI

Liver electrolyte, water, and solids

Number	Days	Per 100 grams of fat-free solids							
		H_2O	Cl	Na	K	P	N	Fat	Glycogen
		grams	*mM*	*mM*	*mM*	*mM*	*grams*	*grams*	*grams*
		Fasting Controls							
1	0	290 ±7.5	11.0 ±0.7	10.4 ±1.3	35.9 ±2.3	40.5 ±2.7	12.7 ±0.6	9	
2	1	296	13.3	10.5	36	41	13.9	11	3.4
3	2	280	13.3	13.1	38	44	14.3	24	0.8
4	7	324	11.6	9.2	38	44	13.2	6	3.1
		Fasting DOCA							
5	0	334 ±18	13.4 ±0.8	14.7 ±1.3	39.9 ±3.2	44.8 ±3.1	14.2 ±0.5	12	3.7
6	1	256	11.0	10.6	34	41	13.5	4	0.1
7	2	279	11.9	11.2	36	42	14.3	14	0.4
8	7	289	12.2	12.5	25	41	14.0	7	1.2
		Normal 20,000 feet							
9	1	263	11.2	9.2	30	35	12.3	5	
10	2	332	12.3	9.4	33	37	12.5	10	
11	7	265	13.1	9.7	33	37	13.1	15	
		DOCA 20,000 feet							
12	1	263	12.8	11.6	33	39	13.2	3	1.5
13	2	286	14.4	10.7	35	41	13.9	14	
14	7	293	13.7	11.8	35	39	13.5	20	12.1
		Low K 20,000 feet							
15	1	262	12.0	10.8	36	38	12.2	14	12.9
16	2	267	10.9	10.6	36	41	12.4	20	7.8
17	7	270	13.6	11.8	36	43	12.9	15	4.3
		Normal 25,000 feet							
18	1	277	13.1	12.9	36	38	13.4	12	1.7
19	2	268	12.6	9.8	36	41	12.9	13	12.7
20	7	264	14.4	8.5	34	35	13.5	7	4.8
		Low K 25,000 feet							
21	1	237	8.7	9.6	31	47	15.1	21	1.4
22	2	313	14.0	12.6	35	49	15.4	37	8.6
23	7	269	14.5	10.4	27	39	12.8	7	6.4

When normal rats are exposed to low atmospheric pressures, the changes in liver composition are for the most part within the variations of the fed and fasted controls. Water is high in the livers of those exposed for 2 days at 20,000 feet. Note the high fat at both levels, probably chiefly an effect of poor appetite with the shift of liver metabolism to combustion of fat. The high liver glycogen after 2 days at 25,000 feet is evidence of the Evans (13 to 16) phenomenon. In all groups, the chloride tends to be higher than sodium and this difference is exaggerated in the rats kept at 25,000 feet. Since at this pressure there is increase in serum chloride and no change in serum sodium, the change in tissue chloride and sodium cannot be interpreted as indicating any fundamental change in the intracellular phase.

The exposure to low atmospheric pressure of rats receiving DOCA likewise produces no striking change, attributable to anoxemia, in the water and electrolyte pattern of the liver. Liver fat becomes high owing to fasting; and high glycogen

after 7 days at 20,000 feet exhibits the Evans' phenomenon, since these rats were not eating although food was in the cages. The rats on the diet low in potassium show low liver water, Na, and Cl after 1 day at 25,000 feet. This finding indicates loss of extracellular water. The high glycogen after 1 day at 20,000 feet is attributable to anoxemia, since the rats do not eat during the first day of exposure. After 7 days at 25,000 feet, there is decrease in concentration of liver potassium. Since a similar finding occurs in fasted rats treated with DOCA, the phenomena probably should be attributed to fasting rather than anoxemia. Thus, there are striking changes in liver size and increases in certain organic constituents. The increase in fat is related to poor appetite; the increase in glycogen, to deficit of carbon dioxide. However, no changes in water and electrolyte are directly attributable to anoxemia.

COMMENT

Certain of our findings suggest that overactivity of the adrenals is a feature of the reaction of rats to low atmospheric pressure. While the histological data do not prove that the disappearance of cortical lipid is brought about by an increased rate of discharge rather than a decreased rate of production, the fact that the histological changes are demonstrable within 2 hours suggests that increased rate of discharge is the correct explanation. Houssay, *et al.* (17), Selye (18), Dosne and Dalton (19), and Sarason (6) also attributed decrease of cortical lipids to activation of the cortex. The degree of distress produced by low atmospheric pressures was least in the rats fed a normal diet, intermediate in those fed the diet low in potassium, and greatest in those receiving DOCA. The extent of lipid depletion is, thus, proportioned to the evidences of distress. This relationship between distress and depletion of cortical lipids is also supported by the fact that, in each group, greater depletion of lipid was found in the rats exposed to 25,000 feet than in those exposed to 20,000 feet. If increased discharge of cortical lipid continues during long periods of exposure to low atmospheric pressure, production is so increased as to enable the adrenals to appear normal within a week.

Some previous work has suggested that enlargement of the adrenals accompanies exposure to low atmospheric pressure. Armstrong and Heim (20) report adrenal enlargement in rabbits exposed daily for 4 hours, over a period of 5 weeks, to a simulated elevation of 18,000 feet. Dohan (21) and Langley and Clarke (22) report enlargement after short exposures but their findings are dubious because of variations in the size of the animals and because losses in body weight were not properly taken into consideration. Cole and Harned (23) showed that adrenal weight varies directly with body weight and Mulinos and Pomerantz (5) and Jackson (4) found that adrenals maintained their size relatively well during inanition. From a review of the literature, Tepperman and Engel (24) thought cortical hypertrophy occurred under circumstances leading to increased metabolism of proteins. Sundstroem and Michaels (25) found no adrenal enlargement in rats except those exposed to pressures lower than 300 mm Hg. Enlargement of the adrenals is apparently not a necessary feature of the adjustment in relatively short time experiments, involving pressures at which acclimatization is possible.

The accumulation of sodium within the muscle fibres of rats exposed to low atmospheric pressure suggests overactivity of the adrenals. Rise in intracellular sodium and loss of intracellular potassium are readily produced by repeated injections of desoxycorticosterone acetate (1, 26, 8) and can be produced by cortical extract and some other related compounds (27). In these cases, rise in sodium is accompanied by fall in potassium. Only accumulation of sodium was demonstrable in the present experiments. Since injection of desoxycorticosterone acetate at low atmospheric pressure exaggerated the retention of intracellular sodium without leading to greater reduction in muscle potassium, overproduction of cortical hormone may also be the explanation of the accumulation of intracellular sodium in experiments involving no injection of cortical compounds.

Whatever the explanation of the mechanism of the muscle changes, other factors are probably more important than the adrenals in bringing about most of the electrolyte changes in the body as a whole. First, adjustment of acid-base equilibrium is carried out by renal excretion of sodium

and this process goes on in much the same way in our experiments, in the presence of potassium deficit or under the influence of injections of desoxycorticosterone acetate, as in normal rats. The cortical hormone can, therefore, play no primary role in this process. Second, sodium and potassium are excreted owing to fasting and our experiments indicate that fasting at sea level and exposure at 25,000 feet with its accompanying refusal of food lead to the same change in muscle composition. Our experiments give no data which would indicate that the adrenals play a rôle in this process. It should be pointed out that the kidneys are unable completely to reabsorb potassium from the glomerular filtrate and hence deficits of body potassium can be produced (*1*) by diets low in potassium in animals with normal kidneys (28, 1), and (*2*) by normal diets when the kidneys are under the influence of excessive amounts of desoxycorticosterone acetate or cortical extract (1, 26). Since fasting frees large quantities of body potassium, the low muscle potassium which was found after 7 days of fasting or 7 days at 25,000 feet is probably associated with increased rate of renal excretion of potassium. This assumption is supported by the fact that addition of KCl to the drinking water prevented the loss of muscle potassium in rats exposed for 1 week at 25,000 feet. This change of renal function could be produced by increased adrenocortical function. Since the same phenomenon did not develop at 20,000 feet, fasting must be superimposed on anoxemia to change the renal excretion of potassium.

Alkalosis apparently plays no rôle in the changes in muscle composition since Yannet (29) found no change in intracellular sodium or potassium in alkalosis due to low serum chloride and high bicarbonate.

Because of the crucial position of the liver in metabolism, evidences of disturbances in this organ are particularly important. Recent work in progress in this laboratory has shown that, within 1 to 2 hours after bleeding rats sufficiently to produce "irreversible shock," the liver exhibits changes in electrolyte composition indicating alteration of the normal distribution of Na and K. This is manifested first by a disappearance of the excess of Cl over Na and then by a decrease in potassium and increase in sodium. During the exposure of rats to low atmospheric pressures, analogous changes are not discovered. This finding is further evidence that the liver is not as sensitive to arterial as to venous unsaturation.

The data should not be interpreted to indicate that the liver is unaffected, for the changes in liver size are definite but not reflected in changes in water and electrolytes per unit of fat-free solids. Yannet (29) found, in cats, no changes in liver water and electrolyte, attributable to alkalosis. Furthermore, water and electrolyte of the liver per unit of fat-free solids show no variations with loss of extracellular electrolyte from the body (9), with repeated injections of desoxycorticosterone acetate (8), or with feeding of diets low in potassium. Thus, the liver cells seem able to protect their internal composition from certain alterations of their environment, such as changes in concentration of extracellular electrolyte, blood pH, and arterial unsaturation. Nevertheless, the liver undergoes changes in organic composition, such as increase in fat with fasting, which explains the fat changes reported in this study, and increase in glycogen accompanying exposure to low atmospheric pressure, which is illustrated much better in previous studies than in our data (13, 15, 16). The increase in size does not seem to be dependent on chronic passive congestion—at least histological sections do not confirm this supposition. Perhaps the variations in liver composition (30) are so great with the various changes in metabolic activity under normal circumstances that alterations in water and electrolyte are overshadowed in most pathological conditions. On the other hand, changes in liver water and electrolyte may not develop until a more or less complete metabolic breakdown occurs. The latter state, of course, would be found only shortly before death.

From a practical point of view, great importance should be attached to the evidence that exposure to low atmospheric pressure aggravates the tendency of rats receiving desoxycorticosterone acetate to develop myocardial lesions. That this phenomenon is connected with accelerated loss of potassium is indicated by work in progress in our laboratory. It has been shown that the combination of a diet low in potassium with injections of desoxycorticosterone produces demonstrable histological lesions in the heart in 4 to 7 days, whereas either procedure alone takes more than 2 weeks.

The exaggeration of the accumulation of intracellular sodium in muscle of rats receiving desoxycorticosterone acetate while being exposed to low atmospheric pressure may also be an undesirable effect of this steroid. Apparently, at low atmospheric pressures, excessive response to the renal factor of the cortical hormone is not desirable and may even be dangerous. This conclusion does not exclude the possibility that adjustment to low atmospheric pressures may be promoted by an excess of the glycogenic factor of the adrenal cortex. However, the proof for such an advantage can hardly be based on restoring the deficiencies of adrenalectomized animals but must come from evidence that animals having no deficiencies do better with an amount of hormone that is in excess of that which such animals can produce.

SUMMARY

Rats were exposed to atmospheric pressures equivalent to elevations of 20 and 25 thousand feet. Three groups were studied: (*1*) normal rats, (*2*) rats on a diet low in potassium, and (*3*) rats which received desoxycorticosterone acetate for 14 days before as well as during exposure to low atmospheric pressure.

Histological evidence of depletion of adrenal cortical lipid was found starting within 2 hours and reaching a maximum about the 48th hour of exposure. Recovery of cortical lipid is apparently complete in 7 days, even while the rats are continuously exposed to a level equivalent to 25,000 feet. Fasting alone does not produce similar changes. The extent of the depletion of lipid varies directly with the evidences of distress, being greatest in rats receiving desoxycorticosterone acetate, intermediate in those on the diets low in potassium, and least in those on the normal diet. Changes in adrenal size are not statistically demonstrated if adrenal weights are related to initial body weights. An exception is found in that moderate enlargement is demonstrable in rats on a diet low in potassium, after exposure for 1 or 2 days at 25,000 feet.

The tendency of desoxycorticosterone acetate to produce cardiac lesions is aggravated by exposure to low atmospheric pressures.

Decrease in muscle potassium is found in rats fasted for 7 days or kept at a pressure equivalent to an elevation of 25,000 feet for 7 days. This finding is considered a result of fasting. On exposure to low atmospheric pressure, normal rats and those receiving DOCA show an increase in intracellular sodium of muscle. Anoxemia seems to promote this change. No decrease in muscle potassium accompanies this increase in intracellular sodium.

Changes in liver composition (except increase in liver glycogen) can be related to fasting but not to the direct effects of low atmospheric pressure. On the other hand, exposure to low atmospheric pressure frequently induces enlargement of the liver.

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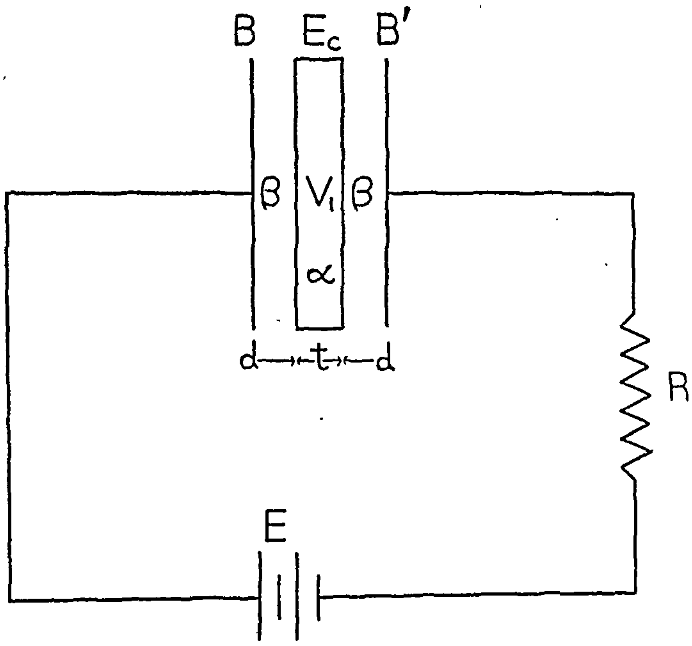


FIG. 2
For explanation see text.

in the electrostatic field of the condenser plates B and B'. The capacitance, C, of the condenser is a function of the volume V_1 of α and the volume V_2 of β, and of the dielectric constant of each substance. The total volume between the plates is constant and is determined by the area of the plates and the distance between them.

When the dielectric constant of α is large in comparison with that of β, as for example in the case of water and air, it is readily shown from elementary electrical theory that

$$C = C_0(1 + V_1/V) \qquad (1)$$

where C denotes the capacitance, C_0 the capacitance when V_1 is zero, or when the entire space is occupied by β, V_1 is the volume of α, and V is the total volume between the plates, a constant.

The capacitance is found to be approximately a linear function of the volume of the liquid dielectric. Reasoning along the same lines for a system of any number of dielectrics in series, the volume of 2 varying in such a manner that their sum is constant, the same conclusion is reached, that the capacitance is a linear function of the variable volume.

This reasoning provides a basis for the provisional assumption that, in intact animals or man, the capacitance change during the cardiac cycle is proportional to the change of volume of the heart. The necessary premises are that the variable parts of the volume are the volume of blood in the heart (high dielectric constant) and the volume of air in the lungs (low dielectric constant). The volumes and configurations of all other structures between the plates are assumed constant.

Having arrived at an approximate relation between the capacitance and the variable volume of the model, it is necessary to consider the relation between capacitance and E_c, the difference of potential across the condenser plates (Figure 2).

The fundamental equation between C and E_c is

$$E_c = q/C \qquad (2)$$

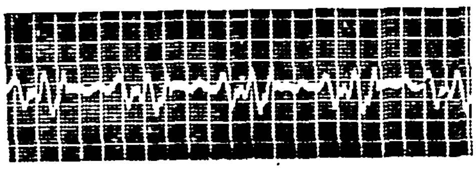
where q is the charge on the plates expressed in coulombs, C the capacitance in farads, and E_c the potential difference in volts. When q is maintained constant, E_c varies inversely as C. In general, one is not justified in regarding q as constant, except for certain limiting cases. Whenever E_c changes above or below its equilibrium value E, the voltage of the battery, the condenser will tend either to discharge or to be charged, q varying in accordance with the laws of condenser discharge.

From the theory of condenser discharge, three conditions for minimal charging or discharging during any capacitance change can be deduced. First, during an instantaneous change of capacitance, q may be assumed constant, for discharge or charge is a time process. Second, when the resistance R of the circuit is very large, q will tend to remain constant. Third, the tendency to charge or discharge will be minimal when E_c approaches E.

For each of these cases, the potential difference of the condenser plates varies inversely as the capacitance, which in turn varies linearly with the volume of the heart, according to our hypothesis. Referring to Figure 1, it is evident that the potential of one condenser plate, B, is constant. The variable part of the potential is that of the plate B', which is connected directly to the grid of the amplifier tube. The grid voltage determines plate current and the voltage drop across the leads of the recording oscillograph.

Actually, the case of cardiac activity in intact animals or in man must be much more complex than that of any simplified theoretical model which might be postulated, and the relations that have been deduced cannot be assumed to hold in prac-

tice until many more experimental data have been obtained under a wide range of conditions. In studies on man, one is dealing not only with changes of the heart volume but also with changes of configuration and position. The field between the plates includes not only the heart but also varying sections of the great arteries and veins. and of the pulmonary circulation. In this case, the variable volume is surrounded by a sheath of conducting media, which shields to a large extent the volume and dielectric changes which are to be recorded. These factors vary in every case and would of course be extremely difficult to evaluate in a general manner. Until a large mass of data



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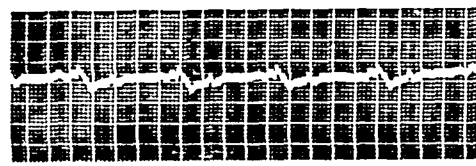

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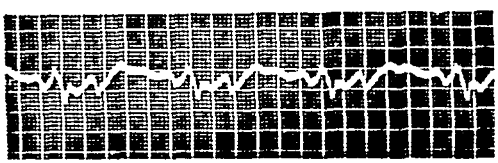

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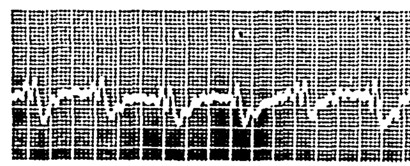

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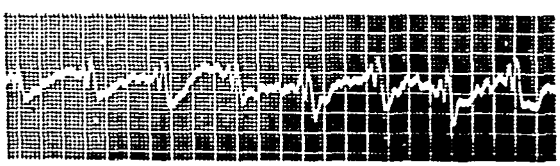

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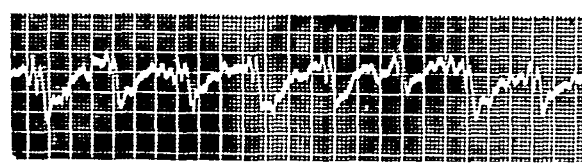

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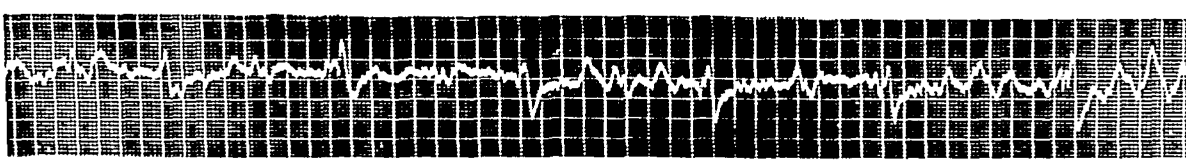

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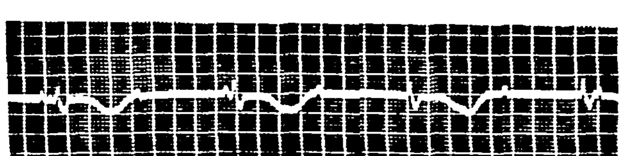

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has been studied, a formulation of the relation between capacitance changes and volume changes will not be attempted.

RESULTS

Records have been obtained on about 20 subjects with normal hearts and on a few subjects with heart disease. Some representative records are presented in Figure 3. These samples include most of the types of record that have been obtained. In many cases, electrocardiographs have been obtained within a few minutes after the condenser cardiograph records were taken. In all of these cases, no significant difference between the heart rates as measured by the two methods could be observed. In one case, the rate was determined by the intervals between the R waves, and in the other case, by the intervals between the major deflections of the condenser cardiograph records. In some cases, electrocardiographs were taken immediately before or after the condenser cardiographs without stopping the camera. In such cases, the R waves were found to correspond in time very closely to the major deflections of the condenser cardiographs, when the time intervals were extrapolated.

Somewhat similar records have been obtained by Atzler and Lehmann (1) and by Rosa (2), who employed a different type of circuit from that described in this communication. They have employed alternating current of radio frequency across the electrodes. The amplifier described in the present paper is much simpler and would seem to be more generally applicable, since it is of a simple direct current type.

No attempt will be made at present to interpret any of the records, except for the statement that, in most of them, the characteristic pattern of each cycle is quite constant, and that, in general, the records tend to be fairly reproducible for a given subject. Obviously, a thorough interpretation would be very difficult because of the many complicating and variable factors that probably affect the results.

One case, however, deserves particular attention. This is the record (Figure 3, No. 7) of a patient with complete heart block. Here, the ventricular rate, about 30 per minute, is found to correspond closely with that of the electrocardiograph (Figure 3, No. 8), and a rhythm of approximately twice this frequency, probably auricular, can be distinguished. Study of a large number of various types of clinical cases might be expected to be of value in interpreting the features of the condenser cardiograph records.

SUMMARY

1. A direct current amplifier for recording capacitance changes is described, and its application to the study of cardiac activity is indicated.

2. It is shown theoretically that, as first approximations, capacitance varies directly as the dielectric constant and the volume, and that the potential difference across the condenser plates varies inversely with the capacitance.

3. Records of cardiac activity in human subjects are presented and discussed.

4. It is pointed out that, due to a large number of complicating factors, the results can be regarded as having only qualitative and empirical significance.

The author is indebted to Dr. Johnson McGuire for his interest and encouragement.

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THE CHLORIDE EXCRETION TEST (CUTLER–WILDER–POWER TEST) IN ASTHENIA

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The clinical picture of Addison's disease is well recognized and understood. It is very likely, from analogy with other diseases or deficiencies, that milder cases occur which, for the most part, are not recognized as such. However, the diagnosis of "mild hypoadrenia" made by a number of authors (1 to 5) rests on clinical impression and on the therapeutic effect of substitution therapy alone. These criteria for diagnosis are insufficient and the procedure of treatment is costly. It was therefore decided to investigate the adrenal cortical function in asthenic patients.

MATERIAL AND METHODS

Fifty patients have been studied. Of these, 27 complained of varying degrees of asthenia. Carbohydrate metabolism was studied by means of the glucose tolerance test, 100 grams of glucose being given orally. Blood sugar determinations were done by Benedict's method. The values reported are therefore "true sugar values." All patients were given a standard mixed diet for at least 5 days prior to testing the glucose tolerance.

Orthostatic blood pressure regulation was tested in the majority of cases.

As a test for adrenal insufficiency, the chloride concentration in urine, following a test diet poor in Na, rich in K, has been employed (Cutler, Power, and Wilder (6)). This test will be referred to as the Cutler test.

OBSERVATIONS AND RESULTS

Since in Addison's disease there is a high concentration of Cl in the urine under the conditions of the Cutler test (225 mgm. per cent and above), it was thought that less pronounced adrenal insufficiency, or "mild hypoadrenia," might be recognized through a Cl concentration at or above the upper limit of normal.

Our findings are tabulated in Table I. As will be discussed later in this paper, the Cutler test was performed repeatedly in some of the cases. Each case is listed only once in this table, under the highest excretion observed.

Studies similar to those reported in this paper were performed in a number of cases which at first, on the basis of their personality and the nature of their complaints, impressed us as possibly belonging to this group. In the course of clinical studies, some organic disease was found or suspected and such cases were consequently not included in this report, despite the fact that the organic findings did not always seem responsible for the complaints or failed to explain them adequately.

The table shows that 23 out of 50 patients did not complain of asthenia. Such patients with vague complaints, in some instances with low blood pressure, in which careful clinical observation failed to reveal any physical disorder, seemed to us to be more adequate "controls" than normal healthy individuals.

It will be seen from Table I that out of 27 patients complaining of asthenia, 11 had a Cl concentration of more than 125 mgm. per cent, and 3 of these, a Cl concentration of more than 225 mgm. per cent. Of the 22 patients not definitely complaining of asthenia but resembling the asthenia cases in some respects, only 3 had a concentration of more than 125 mgm. per cent.

Na excretion was determined together with the Cl excretion in 7 cases. The three highest values observed are given in the table.

Since a Cl concentration of 225 mgm. per cent and above has been considered indicative of Addison's disease, the case histories of the 4 cases showing such high Cl excretions are given in brief abstract.

Case 15. A Polish woman, aged 58 years, was admitted with complaints of cramps in stomach, belching, loss of appetite, weakness. Her complaints had lasted many years and she had been treated with various drugs. Twelve years ago, cholecystectomy had been performed in the hope of relieving her. The operation failed to help. At present, the possibility of postoperative adhesions was considered, but physical and roentgenographic examinations were entirely normal. She was

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TABLE I

Cl excretion during 4-hour period of third day of Cutler test, in relation to clinical manifestations

Range of of Cl excretion*	Number of cases		Positive psychiatric findings	Hypotension	Orthostatic blood pressure drop	Flat glucose tolerance curve
mgm. per 100 cc.						
23 to 125	36	"Asthenia" 16	13	9	0	5
		No "Asthenia" 20	12	3	1	6
125 to 225	10	"Asthenia" 8**	6	4	1	1
		No "Asthenia" 2***	2	2	0	1
Over 225	4	"Asthenia" 3****	2	2	0	3
		No "Asthenia" 1	1	1	0	1

* During 4-hour period on third day of Cutler test.
** One case: Sodium excretion 135 mgm. per 100 cc.
*** One case: Sodium excretion 87 mgm. per 100 cc.
**** One case: Sodium excretion 114 mgm. per 100 cc.

5 feet tall and weighed 98 pounds. Psychiatric diagnosis by Dr. B. L. Keyes and Dr. R. A. Matthews, to whom we are indebted for the psychiatric examination of all cases of this study, was anxiety neurosis with conversion. A glucose tolerance test showed: fasting, 72 mgm. per cent; 1 hour after 100 grams of glucose (orally), 81 mgm. per cent; 2 hours, 114 mgm. per cent; 3 hours, 102 mgm. per cent; 4 hours, 77 mgm. per cent; 5 hours, 98 mgm. per cent; 6 hours, 80 mgm. per cent. Her blood pressure in the recumbent position was 94/62, and in the erect position, 100/64. At times, her blood pressure while recumbent was as high as 150/80. The Cutler test showed that during the 4-hour period of the third day, she excreted 250 cc. urine, with a Cl concentration of 228 mgm. per cent and a Na concentration of 114 mgm. per cent.

In spite of these findings, it was not believed that this patient had Addison's disease. She was dismissed with the diagnosis of psychoneurosis.

Six months later she was again studied. Her condition was unchanged. Her glucose tolerance curve was again "flat": fasting, 69 mgm. per cent; 1 hour after 100 grams of glucose (orally), 75 mgm. per cent; 2 hours, 93 mgm. per cent; 4 hours, 58 mgm. per cent; 5 hours, 68 mgm. per cent; 6 hours, 67 mgm. per cent. The Cutler test showed that during the 4-hour period of the third day, she excreted 820 cc. urine, with a Cl concentration of 77 mgm. per cent.

Fifteen months later another study was made. She had gained 30 pounds, but her condition was otherwise unchanged. A glucose tolerance test showed: fasting, 80 mgm. per cent; ½ hour after 100 grams of glucose, 122 mgm. per cent; 1 hour, 117 mgm. per cent; 2 hours, 102 mgm. per cent. The Cutler test demonstrated that, during the 4-hour period of the third day, 740 cc. urine were excreted, with a Cl concentration of 14 mgm. per cent.

Case 19. An Italian woman, aged 38 years, was admitted with complaints of nervousness, weakness, and pains and aches in her arms and legs. The physical examination was entirely negative. She weighed 102 pounds; her blood pressure was 120/80 in the recumbent position, 118/82 in the erect position; B. M. R. was plus 8. The glucose tolerance test showed: fasting, 70 mgm. per cent; 1 hour after 100 grams of glucose (orally), 54 mgm. per cent; 2 hours, 92 mgm. per cent; 3 hours, 71 mgm. per cent; 4 hours, 66 mgm. per cent; 5 hours, 66 mgm. per cent; 6 hours, 66 mgm. per cent. The Cutler test showed that during a 4-hour period of the third day, 140 cc. of urine were excreted, Cl concentration 311 mgm. per cent. This test was repeated several days later. The urine volume was 140 cc., and the Cl concentration, 411 mgm. per cent. Psychiatric diagnosis: psychoneurosis.

Patient was re-examined one year later. She had received no glandular substitution therapy. An appendectomy had been performed elsewhere since the above mentioned studies were made. She had tolerated the operation well. Her general condition was unchanged. She refused readmission for further study.

Case 21. A Jew, aged 40 years, was admitted with complaints of weakness and exhaustion, coming in attacks during the past 7 months. Physical examination was entirely negative. Psychiatric examination did not reveal any psychoneurosis. His weight was 134 pounds; his height, 67½ inches. His blood pressure was 96/54 in the recumbent position and 102/60 in the erect position. A glucose tolerance test showed: fasting, 93 mgm. per cent; 1 hour after 100 grams of glucose (orally), 108 mgm. per cent; 2 hours, 77 mgm. per cent; 3 hours, 85 mgm. per cent; 4 hours, 71 mgm. per cent; 5 hours, 67 mgm. per cent; 6 hours, 71 mgm. per cent; B. M. R. was plus 1. The Cutler test showed that during the 4-hour

period of the third day, the urine excretion and Cl concentrations were 110 cc., Cl 228 mgm. per cent; 840 cc., Cl 43 mgm. per cent; and 420 cc., Cl 61 mgm. per cent.

The patient was questioned one year later. He had not consulted his physician because he had felt well.

Case 38. A Jewess, aged 33, was admitted with complaints of dizziness and palpitations. Physical examination, roentgenogram, and electrocardiogram were entirely normal. Her height was 64 inches; her weight, 138 pounds. Blood pressure was 110/75, but at times up to 130/80. Results of a glucose tolerance test were: fasting, 68 mgm. per cent; 1 hour after 100 grams of glucose (orally), 106 mgm. per cent; 2 hours, 100 mgm. per cent; 3 hours, 70 mgm. per cent; 4 hours, 76 mgm. per cent; 5 hours, 81 mgm. per cent; 6 hours, 79 mgm. per cent. B. M. R. was plus 7. The Cutler test showed that during the 4 hours of the third day, 100 cc. of urine were excreted with a Cl concentration of 225 mgm. per cent. Psychiatric diagnosis: psychoneurosis.

Patient was next seen 1½ years later. Her condition was unchanged. Because of amenorrhea, a gynecologist had given her estrogenic hormones and thyroid, both of which were tolerated well, but failed to influence her condition. She was readmitted to the hospital in order to repeat the Cutler test. She then excreted during the 4 hour period of the third day, 760 cc. urine with a Cl concentration of 76 mgm. per cent.

It is clear from the observation of these 4 patients, over a period of from 1 to 2 years, that none of them was a case of Addison's disease. In addition, it may be pointed out that one of these patients (Case 19) was operated upon successfully without any of the precautions and procedures necessary in Addison's disease. Another (Case 38) showed no ill effects from thyroid medication received elsewhere, whereas patients with Addison's disease react unfavorably to thyroid therapy. The variation of Cl excretion on repeated testing will be discussed later.

The following observation led us one step further. In one case, some doubt was raised as to whether the regime during the test had been properly and accurately maintained. The Cl excretion had been excessively high (341 mgm. per cent Cl). The test was repeated one week later and the Cl excretion was low (7 mgm. per cent Cl). No information is available as to the variation of the Cl excretion in the same individual at different times. Willson and associates (7) found the Cl excretion of a patient suffering from Addison's disease, on whom the test was performed at different times, to be remarkably constant. When we began to test our patients more than once, at short intervals, a considerable variation of Cl excretion in the Cutler test was observed. Since the majority of our patients were young women, we thought of an influence of the menstrual cycle. Thorn and his associates (8) have shown the sex steroids to induce salt and water retention when injected into dogs. In human females, this salt and water retaining effect on the individual's own estrogenic hormone may be potent enough to produce premenstrual weight increase and edema (9). It was consequently thought possible that the result of the Cutler test might vary considerably in different phases of the menstrual cycle.

Five healthy female volunteers were therefore subjected to the procedure of the Cutler test every week for one month. The regime of these 5 girls differed from that of our patients inasmuch as they were not hospitalized or at bed rest during the test periods. They all continued their activities as technical assistants in the laboratories. However, their meals were prepared by the diet kitchen in the hospital. All drinking water was

TABLE II

Cutler tests performed at weekly intervals on normal healthy young girls

	Weight	Test 1		Test 2		Test 3		Test 4	
		Cl	Urine volume	Cl	Urine volume	Cl	Urine volume	Cl	Urine volume
	kgm.	*mgm. per 100 cc.*	*cc.*	*mgm. per 100 cc.*	*cc.*	*mgm. per 100 cc.*	*cc.*	*mgm. per 100 cc.*	*cc.*
M. S.	57.3	*23	720	38	1240	31	910	35	900
K. H.	51.8	20	760	*28	460	20	630	26	350
K. E.	63.0	32	870	36	1000	*31	960	35	1000
J. L.	51.8	*66	340	31	930	88	300	30	460
L. B. (1)	61.6	*28	930	45	620	40	1180	*	

Cl: Concentration and urine volume during 4-hour period on third day of Cutler Test.
* Menstruation occurring between respective tests.
(1): L. B. menstruated irregularly. Menstruations three weeks apart during observation period.

TABLE III

Cl excretion and urine volume during 4-hour period of third day of Cutler Test. Results of repeated tests

Case number	Test 1	Test 2	Test 3	Test 4
15	228 mgm. per cent (250 cc.) (January 23, 1941)	77 mgm. per cent (820 cc.) (June 26, 1941)	14 mgm. per cent (740 cc.) (October 6, 1942)	
19	311 mgm. per cent (140 cc.) (May 12, 1941)	411 mgm. per cent (140 cc.) (May 20, 1941)		
21	228 mgm. per cent (110 cc.) (June 28, 1941)	43 mgm. per cent (840 cc.) (July 7, 1941)	61 mgm. per cent (420 cc.) (July 12, 1941)	
24	104 mgm. per cent (370 cc.) (August 28, 1941)	25 mgm. per cent (600 cc.) (September 15, 1941)	74 mgm. per cent (780 cc.) (October 20, 1941)	
25	197 mgm. per cent (570 cc.) (August 9, 1941)	45 mgm. per cent (740 cc.) (August 27, 1941)	33 mgm. per cent (450 cc.) (November 10, 1941)	106 mgm. per cent (485 cc.) (September 8, 1942)
27	202 mgm. per cent (80 cc.) (July 20, 1942)	86 mgm. per cent (330 cc.) (July 30, 1942)		
30	86 mgm. per cent (500 cc.) (August 30, 1942)	157 mgm. per cent (390 cc.) (September 25, 1942)		
37	86 mgm. per cent (300 cc.) (September 16, 1941)	93 mgm. per cent (385 cc.) (September 22, 1941)		
38	225 mgm. per cent (100 cc.) (July 7, 1941)	76 mgm. per cent (760 cc.) (October 10, 1941)		
41	*341 mgm. per cent (60 cc.) (July 2, 1941)	7 mgm. per cent (260 cc.) (July 7, 1941)	118 mgm. per cent (230 cc.) (July 14, 1941)	63 mgm. per cent (610 cc.) (July 21, 1941)
45	133 mgm. per cent (45 cc.) (February 5, 1942)	68 mgm. per cent (500 cc.) (February 19, 1942)		
50	172 mgm. per cent (60 cc.) (December 2, 1941)	112 mgm. per cent (220 cc.) (March 30, 1942)		

* This first test was possibly due to an error in procedure (see page 31). This case is therefore listed in Table I under the range 23 to 125 mgm. per cent.

distilled. On the days between the tests, a mixed diet was taken. Table II gives the results. It can be seen that there is little variation in the excretion of these normal individuals. None of them suffered from premenstrual edema or premenstrual tension. It is possible that in women suffering from premenstrual tension and/or edema, the disturbed salt-water metabolism might influence the results of the test. It is, however, obvious that in the normal subject, the rhythmic changes in sex steroid production are not capable of overcoming the rigorous conditions of the test.

In the further course of our studies, we found considerable variation of Cl concentration in the tests performed on post-menopausal women and in male individuals. This further emphasizes the fact that this variation is independent of the ovarian cycle.

In Table III, the variations observed on repeated tests are listed. None of the changes could be attributed to any form of treatment. As a matter of fact, in those instances in which the test was repeated at short intervals during one hospital stay of the patient, no treatment was given during the interval. The changes of Cl excretion observed did not coincide with any change of the clinical picture or of the subjective complaints.

The considerable variability of intermediate metabolism is not limited to Cl retention. A case in point is Case 25.

S. L., a Jew, aged 26, complained of extreme weakness and difficulty of breathing and swallowing. He was obsessed by the idea that something was wrong in his chest. Repeated examinations, including roentgenography and laryngoscopy, failed to detect any organic disease.

He was greatly emaciated, weighing 86 pounds (38.7 kgm.), height 63½ inches (154 cm.). His blood pressure varied from 80/60 to 120/80. The Cutler test showed a urine volume, in 4 hours of the third day, of 570 cc. with a Cl concentration of 198 mgm. per cent; several days later it was 335 cc. with a Cl concentration of 46 mgm. per cent. The fasting blood sugars were as follows: November 6, 38 mgm. per cent; November 7, 82 mgm. per cent; November 11, 56 mgm. per cent. His B. M. R. was minus 16; several weeks later, while his condition was unchanged, the B. M. R. was plus 14. The psychiatric diagnosis was psychoneurosis, possibly incipient schizophrenia. Anorexia nervosa was also considered.

Further management of the patient proved impossible. He went from one hospital to another. In the course of one year, following the above mentioned studies, he was admitted to different hospitals four times, including one admission to a psychiatric department. He returned to Jefferson Hospital after one year. His weight had further decreased to 74 pounds. His glucose tolerance was: fasting, 71 mgm. per cent; ½ hour after 100 grams of glucose (orally), 115 mgm. per cent; 1 hour, 115 mgm. per cent; 2 hours, 112 mgm. per cent. The Cutler test showed a urine excretion, during 4 hours of the third day, of 485 cc. with a Cl concentration of 106 mgm. per cent.

This case shows, in addition to variation of Cl concentration in the Cutler test, a very considerable variation of fasting blood sugars.

Glucose tolerance. Seventeen of all our cases showed a flat glucose tolerance curve. Nine of these were asthenia cases. Dorst (10) has previously drawn attention to the flat glucose tolerance curve of patients with neurocirculatory asthenia. It should be emphasized that, both in our cases and in Dorst's series, glucose tolerance was tested by oral administration of glucose. The "flat" curves may be caused by a disturbance of resorption since normal curves were obtained in a few cases following intravenous administration of glucose. In no case did we encounter the exaggerated posthyperglycemic hypoglycemia, characteristic of Addison's disease.

Blood pressure. Blood pressure was low in 21 cases, 15 of these being in the asthenia group. Systolic pressures below 120 mm. Hg were listed as low. Six cases showed clearly hypotensive values (systolic pressure below 100, diastolic pressure below 60). Two patients presented orthostatic hypotension, one of them was in the asthenia group.

DISCUSSION

Cutler and his associates (6) stated that normal persons, as well as patients suffering from diseases other than Addison's disease, do not excrete chloride in excess of 125 mgm. per cent in a 4-hour urine sample collected on the third day of the test. The values in patients with Addison's disease were 225 mgm. per cent or above. Dryerre (11) believes that the determination of Na concentration, under the conditions of the Cutler test diet, gives more satisfactory results than the determination of Cl. Cutler and his associates, having determined both Cl and Na concentration, found changes of both ions to be parallel and therefore advocated the much simpler determination of Cl. Willson, Robinson, Power, and Wilder (7) have recently reported on further experience with this test. They confirm generally the findings published previously by Cutler *et al.* They find the upper limit of normality at 156 mgm. per cent of Cl, and at 85 mgm. per cent of Na, in the urine of the 4-hour period of the third test day.

Stephens (12) reported 2 cases of hypopituitarism in which the Cutler test revealed Cl excretion of a degree indicative of adrenal cortical insufficiency, which is to be expected in such cases.

Thorn, Howard, and Dayman (13) studied 60 patients with pulmonary tuberculosis. In all, the Cl excretion was well within normal range. The 2 with the highest Cl excretion were suspected of having Addison's disease.

In the first report by Cutler, Power, and Wilder (6), the figures for cases without Addison's disease were analyzed. In this series, neither the mean nor the range of Cl concentration of cases of "asthenia" differed from those in other cases without Addison's disease or from those in healthy normal men and women. However, only 6 cases of "asthenia" were examined.

It has been found in this study that a number of asthenic patients excrete chlorides at or above the upper limit of "normality" under the conditions of the Cutler test. Four of these patients excreted chlorides in amounts usually considered diagnostic for Addison's disease. The question may be raised whether this is due to functional impairment of the adrenal cortex. Na and Cl metabolism is influenced by a number of conditions. Chronic infections, especially tuberculosis, neoplasm, and cardiac failure could be ruled out in our cases (13, 14). Inanition is a factor which deserves brief discussion since a number of our patients, though not all, had lost some weight.

Rubin and Krick (15) have found that in partially starved rats, the balance of Cl and Na and of a number of other electrolytes is negative. Mulinos and Pomerantz (16) have shown that starvation in animals produces atrophy of the adrenals and other endocrine glands. It would seem possible that the patients studied in our investigation would lose appetite on the basis of their neurosis. The consequent partial starvation would cause a depression of various glands, including the adrenal cortex. This decreased function would in turn produce various metabolic disturbances and would furthermore decrease appetite, food resorption, and food utilization, thereby increasing starvation. This is the mechanism presumably operating in cases of anorexia nervosa which, in all metabolic functions, simulates pituitary cachexia. High Cl excretion under the conditions of the Cutler test has been found in pituitary cachexia (12). In a case of anorexia nervosa, not included in this report, we found a strongly positive Cutler test. However, we do not feel that the results in our patients can be ascribed to inanition. One reason is that some of the patients showing high Cl excretion did not present any appreciable weight loss and their general appearance was not that of starvation. Secondly, the considerable variations of the Cl excretion, occurring within short periods in some of the individuals, would seem to preclude starvation as a cause of the high Cl excretion.

We can then state that some patients suffering from exhaustion and asthenia, most of them psychoneurotics, respond to the Na privation and K ingestion (test of Cutler and associates) with higher chloride excretion than do normal individuals. In some cases, the magnitude of the Cl excretion is such as is generally considered indicative of Addison's disease. Since these patients did not suffer from Addison's disease, a positive Cutler test may be misleading and cannot be considered proof of Addison's disease. The transient nature of this disturbance in our asthenia cases not suffering from Addison's disease should be stressed.

Low blood pressure was noted in 21 cases, 6 of which were clearly hypotensive. Two patients presented postural hypotension. Postural hypotension may be due to a great variety of factors, (17 to 20). In untreated cases of Addison's disease, the postural hypotension is known to be severe (21, 22, 19).

We believe that our results might be interpreted as indicative of adrenal cortical involvement in some cases of asthenia. This should not, however, imply that we consider these asthenias and neuroses to be caused by adrenal cortical insufficiency. The considerable variability of the Cl balance as shown in several of our cases militates against the assumption of any organic defect of the adrenal cortex. The fact that variations of the chloride retaining power are not accompanied by corresponding changes in the general condition of the patients makes it very unlikely that even functional transitory insufficiency of the adrenal cortex is the *cause* or a *main contributing factor* of the sufferings.

Hypothetically, the explanation of the metabolic findings reported in this paper may rather be sought on the basis of an unstable personality. This instability manifests itself in the psychological sphere as psychoneurosis. The manifestations in the somatic sphere may be very varied.

It seems plausible to assume that the secretion of hormones would participate in this general instability. Variations in blood sugar content may be partly mediated through variations in the output of epinephrine, which in turn is regulated by sympathetic nerve impulses. The adrenal cortex would seem to share the instability of these patients. Whereas the innervation of the adrenal medulla is well recognized, the question of nerve supply of the adrenal cortex is far from settled (23). The variability of output of adrenal cortical hormone might well be part of the personality picture without necessarily being under direct nervous control.

McFarland and Goldstein, in their review of biochemistry of the psychoneuroses (24), come to the conclusion that "in the analysis of the biological reactions of psychoneurotics it appears that their failure to achieve compensation quickly and smoothly may be one of the most important aspects of the illness."

It will readily be seen that the cases discussed in this paper might be subsumed under various headings. Some of them would be described as "types of chétifs" in the French literature. Some would come under the heading of "organ neurosen" of German authors. Reimann's cases of

sympathicotonia and vagotonia seem to resemble a number of our cases (25). Alvarez (26) would regard most of them as "constitutional inadequates." Their endocrine instability is evidently part of their personality, not necessarily its cause. These patients are not suffering from "mild hypoadrenia," and are not mild cases of Addison's disease. These terms would imply that the adrenal cortical insufficiency is the cause of the disease. As mentioned in the introduction, the existence of such a condition might be expected to occur, but we have yet to recognize it.

SUMMARY

1. The chloride excretion test of Cutler, Wilder, and Power has been studied in 50 patients, 27 of whom complained of asthenia, in whom clinical observation failed to reveal any organic disease. Thirty-eight of the patients were found to be psychoneurotic. Cl concentration was 125 mgm. per cent (upper limit of "normal" according to Cutler *et al.*) or above, in 13 cases. The Cl concentration was 225 mgm. per cent or above in 4 patients not suffering from Addison's disease and not suffering from any organic disorder.

2. The chloride excretion test was performed two or more times in 12 cases. A considerable variability in chloride retaining power was found in 11, even when the test was repeated at short intervals.

3. In 5 normal control cases, chloride excretion showed little variation and no influence of the menstrual cycle.

4. It is concluded that some patients suffering from asthenia and exhaustion may temporarily show signs of adrenal cortical insufficiency. Observation of these patients showed that they were not suffering from mild Addison's disease or "mild hypoadrenia." If their inability to conserve Cl is due to lowering of the function of the adrenal cortex, this hypofunction is temporary. It is thought possible that such fluctuations of adrenal cortical function may be part of a general instability of such personalities.

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REGIONAL VARIATIONS IN WATER LOSS FROM THE SKIN OF DISEASED SUBJECTS LIVING IN A SUBTROPICAL CLIMATE[1]

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Clinical examination by usual means indicates variations in the rate of sweating from the skin surfaces of various portions of the body. Attempts to measure these differences quantitatively (Kuno (1), Galeotti and Macri (2)) have confirmed these clinical observations, and the development of more accurate methods (3) has permitted a quantitative evaluation of differences from normal, in a variety of conditions which clinically suggest such changes. Such observations constitute the subject of this report.

METHODS AND MATERIALS

The measurements were made under controlled conditions in an air-conditioned room, maintained at 75° F. $\pm$ 1° and at a relative humidity of 50 per cent $\pm$ 2 per cent. A change from this comfortable environment to hot and humid conditions (105° F. $\pm$ 2° and 75 per cent $\pm$ 2 per cent, respectively) was produced in order to stimulate visible sweating. There were no perceptible currents of air, and, by room design, psychic disturbances were reduced to a minimum.

The technic for measurement of water loss was that used previously (4) in normal individuals. It consists, essentially, of passing dry oxygen through a closed system over a measured area of skin. The quantity of the vaporized water, trapped in aluminum coils by freezing, was calculated by the difference in the weight of the coils before and after the condensation of the water.

Studies were conducted as follows: The subject entered the observation room, where the atmosphere had previously been adjusted to a temperature of 75° F. and a relative humidity of 50 per cent. The patient removed all of his clothing except his underwear, entered a comfortable bed with an innerspring mattress, and covered himself with cotton sheets or a woolen blanket, to suit his comfort. The collecting chambers were sealed in place, and, after a period of approximately 45 minutes, the water loss from the enclosed areas of skin was measured. The water was collected continuously throughout the entire period of study. By the manipulation of stopcocks controlling the flow of the water-laden oxygen and diverting it into one collecting coil or another, the water loss could be separated into 15-minute samples. Thus, collections of sweat for 15-minute periods were made, and then, without the subject's knowledge, the thermostat and humidistat were readjusted, and the room temperature and relative humidity increased to 105° F. and 75 per cent, respectively. About 15 minutes were required for the atmospheric conditions of the room to reach these new levels. During the period of change, and for 30 minutes thereafter, the water loss from the areas of skin under observation was measured so that two 15-minute collections were made while the room was hot and humid. Duplicate or triplicate measurements were made for each part studied.

The rate of water loss from the skin was measured during the winter as well as spring and summer months[2] in New Orleans. The conditions in the room were the same during all seasonal periods. We have previously shown that there is no appreciable difference in the rate of sensible or insensible water loss in these seasons under the laboratory conditions (3). Subjects were used as they became available, regardless of season.

No attempt was made to have the subjects in a postabsorptive state during the determinations. They were advised to eat a light breakfast or lunch, and were then studied about 2 to 3 hours later.

Observations were made on 14 adults displaying a variety of states enumerated below. Eleven were white, and 3 negro. There were 8 females and 6 males, varying in ages from 17 to 56 years.

The areas of skin studied were those over the right and left index finger tips, right and left second toe tips, right forearm (volar surface), anterior surface of chest, epigastrium, umbilical area, anterior surface of the right thigh, right pretibial area, right cheek, forehead, right axilla, left axilla, right leg, plantar surface of the heels of the right and left feet, and the palms of the right and left hands. Three or 4 areas were studied simultaneously.

Comparisons were made with the values obtained for normal adults (3).

RESULTS

In all, 481 separate 15-minute determinations were made on the 14 subjects. In the data, the values given are converted to milligrams per 10 square centimeters of skin area per 15 minutes.

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[1] Aided by a grant from the Rockefeller Foundation.

[2] Average temperature and relative humidity for New Orleans for period of January to March, inclusive, were 55.1° F. and 68.6 per cent, respectively, and for July and August were 83.0° F. and 80.3 per cent.

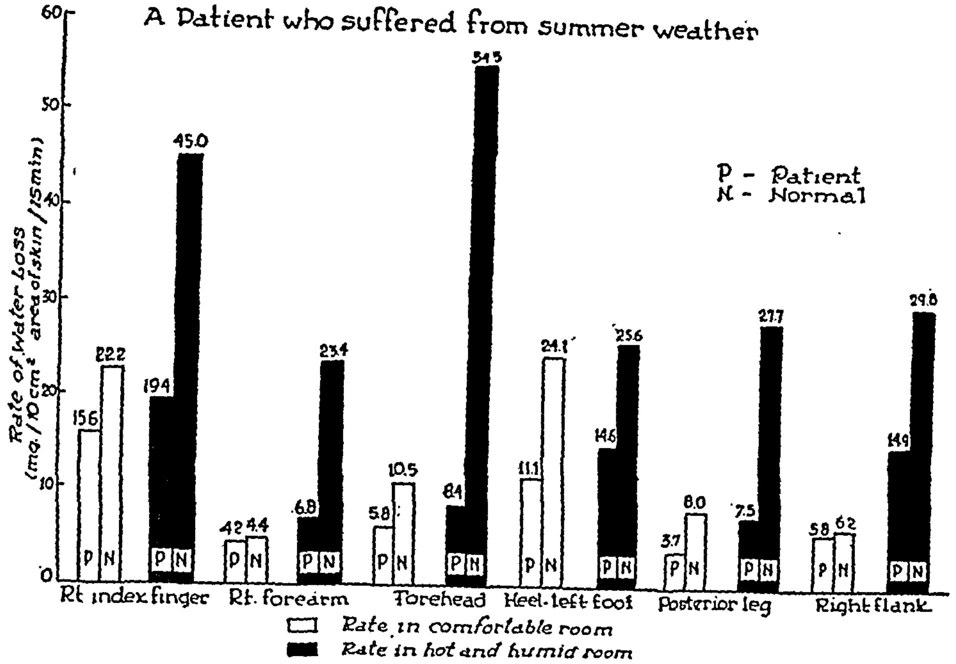

FIG. 4. THE RATE OF WATER LOSS FROM THE SKIN OF SEVERAL AREAS OF THE BODY OF A SUBJECT WHO SUFFERED INTENSELY IN A HOT CLIMATE

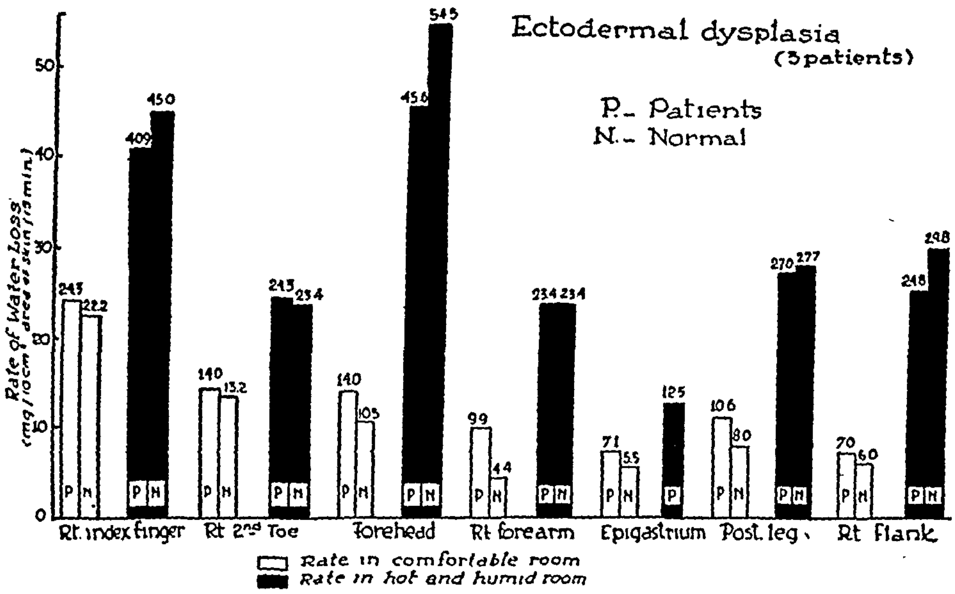

FIG. 5. THE MEAN RATES OF WATER LOSS FROM THE SKIN OF SEVERAL AREAS OF 3 ADULT MEMBERS OF A FAMILY WHO HAD CONGENITAL ECTODERMAL DYSPLASIA

with the sclerodermatous process. In another patient with early scleroderma and Raynaud's disease, values before and after elevation of room temperature, respectively, were for the following areas, 13 and 61 for the right index finger, 11 and 72 for the right forearm, 13 and 79 for the right pretibial area. The marked variations from normal, seen in the previous patient, are not evident here. In a patient with Raynaud's disease with scleroderma, the values were 23 and 40, 17 and 36 for the right and left index fingers, respectively, and 10 and 33 for the right leg. Thus, the figures for this patient fall within the normal range.

A patient with gigantism, acromegaly, and diabetes mellitus complained of dripping wet hands. Her blood Wassermann reaction was positive and her basal metabolic rate, —2 per cent. Values for her right index finger exceeded normal in both environments. They were, respectively, 73 and

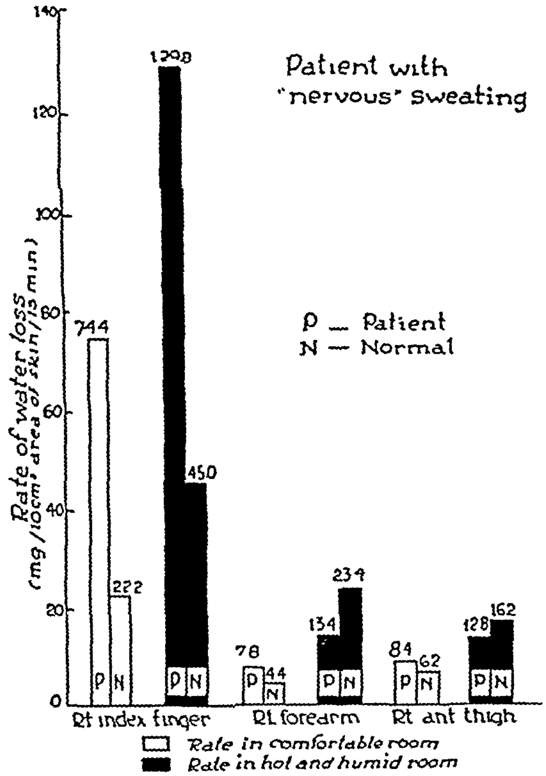

FIG. 6. THE RATE OF WATER LOSS FROM THE SKIN OF SEVERAL AREAS OF THE BODY OF A SUBJECT WHO WAS SUFFERING WITH NEUROCIRCULATORY ASTHENIA AND "NERVOUS" SWEATING

83. Both the right and left axillae showed high values, 20 and 18, in a comfortable room, but values in normal range, 40 and 42 in the hot environment. The forearm and forehead showed similar changes, 8 to 18, and 17 to 35, respectively. In the cheek, both values, 8 and 13, were lower than normal.

A patient with Buerger's disease displayed normal values, 15 to 22 in the right index finger, similar but lower results, 9 and 20, in the second right toe, which, when repeated on another day, were distinctly low, 8 to 10. The values for the forehead fell in normal range, 16 to 71. In the umbilical area, the figures were 7 and 17.

A patient with syringomyelia, studied only in a comfortable room, showed no change from normal. Right index finger, right forearm, and forehead values, were 21, 5, and 12, respectively.

The effects on water loss of a weeping allergic eczema, due to wheat sensitization in a baker, were studied. Values were, for the right palm, 16 and 16; right side of chest, 11 and 12; right index finger, 8 and 25; left heel, 15 and 23; and right heel, 17 and 24. The low initial values in the comfortable room and the failure to develop of the marked rises expected in the hot humid room are evident.

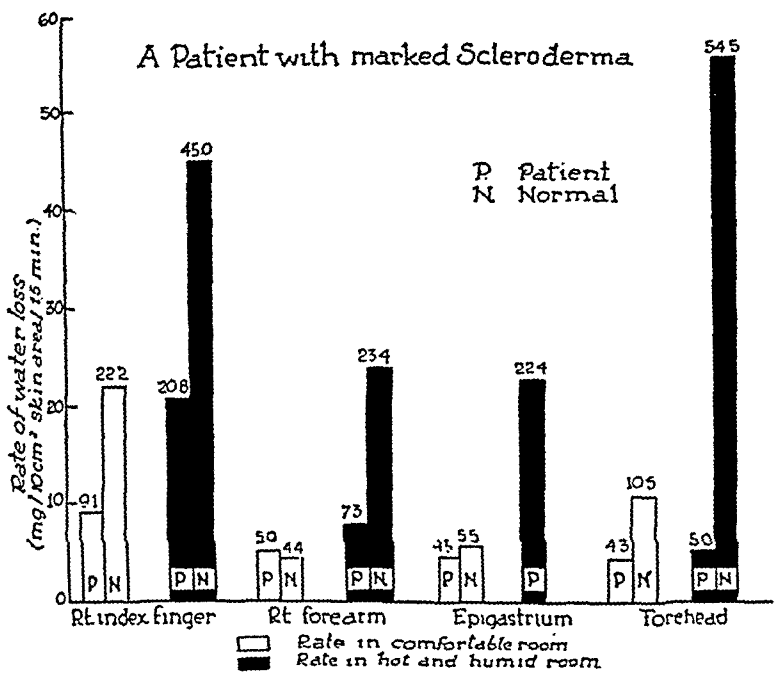

FIG. 7. THE RATE OF WATER LOSS FROM THE SKIN OF SEVERAL AREAS OF THE BODY OF A SUBJECT SUFFERING WITH SCLERODERMA

DISCUSSION

The marked variations in water loss from area to area, from patient to patient, and from time to time in the same patient, must be emphasized. In normal subjects, the variations followed, in general, a definite pattern (3). In descending order, the values for water loss are hands, feet, head, arms, legs, and trunk as seen in Figure 1. In the hot and humid environment, the rate was greatest for the finger tip, axillae, and forehead. It was also noted in the study of normal individuals that the percentage increase, due to transition from the comfortable to the hot room, was as great, or greater, from the skin of the arms, legs, and trunk, as from the finger and toe tips.

Despite the variations in normal individuals from area to area and from person to person, there is evident, as stated above, a general plan or trend of values in the whole group.

The reaction of the thermal stimulus involved all areas studied and presumably involves the entire body. By this means, the organism regulates heat loss as radiation becomes less and less important. Finally, when environmental temperature exceeds body temperature, evaporation becomes the sole means of heat regulation. Variations from normal in this thermal response are to be predicted in hypothyroidism, for in this condition, heat exchange is below normal.

In the patient with the complaint of discomfort in warm weather, there was no obvious evidence to substantiate this complaint until quantitative sweat studies were done and clearly indicated a marked inability to sweat normally with rising environmental temperature. No doubt, other such patients exist. Such physiologic states may account for marked discomfort and inefficiency in men living in subtropical and tropical climates. The abnormal response is not yet explained. Both neurogenic and circulatory control has been postulated to explain the thermal response, but we have not yet been able to demonstrate the importance of either mechanism in this patient.

The disturbed responses in the patients with allergic eczema and scleroderma indicate the possible influence of local dermal factors on water exchange. One would expect that interference with water exchange by local dermal factors might increase water loss through uninvolved, or less involved areas. The values for the patient with scleroderma suggest this possibility. Under any circumstances, these patients demonstrate the effect of local factors on sweating.

Reactions of the sweating mechanism are shown to vary with the stimulus. The general response to the thermal stimulus has just been discussed. Emotional stimuli evoke a different response, increasing the sweat production especially in the hands. This is evident clinically, and our patient with "nervous" sweating obviously falls into this group. In the right index finger in the comfortable room, the values exceeded those of the normal in a hot humid room. The response to heat was excessive, but similar, on a percentage basis, to that of the normal. These results did not hold in the other areas studied. Patients of this type demonstrate regional variations in sweating unlike those in the patients with scleroderma and eczema described above, and resting upon disturbances in the nervous control of the sweating mechanism.

The differences in the response to heat of the finger and of the forearm and the thigh, as compared to each other as well as to the results in normal subjects, would seem to favor the predominance of non-nervous factors in the thermal response in general. However, the group of patients herein studied is so heterogeneous that a series of each type will be necessary before such general conclusions may be drawn.

SUMMARY

A comparison of a group of patients with a variety of conditions affecting the sweating mechanism with a group of normal individuals indicates a much increased variation in the rate of sweating, both insensible and sensible. The conditions vary from those affecting the general controlling factors of the sweat mechanism to local diseases of the skin which appear to interfere mechanically with the process. Obviously, local factors in the sweat glands and skin, so often neglected, may be important in sweat responses.

Variations from normal in nervous sweating and the reactions of these patients to heat-stimula-

tion favor a non-nervous mechanism as the important factor in the thermal response.

Vascular disturbances, such as those found in Buerger's and Raynaud's diseases, which might be expected to affect appreciably the sweating mechanism, had little influence on these reactions.

Quantitative studies of sweat production have been found helpful in establishing objective evidence to account for otherwise inexplicable reactions to heat.

We wish to express our appreciation for the technical assistance and keen interest of Mr. G. Morgavi, who participated in these studies.

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THE EFFECT OF INSULIN ON THE GLUCOSE TOLERANCE OF NORMAL MAN

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INTRODUCTION

Utilization of carbohydrate by the diabetic patient probably differs only quantitatively from that of the normal subject. Many factors which influence carbohydrate tolerance in normal subjects (for example, exercise, emotional disturbances, infections, and the nature of the preceding diet) do likewise in diabetic patients. Any measure which affects the tolerance of the normal subject for carbohydrate merits investigation for its applicability to the treatment of diabetes.

The administration of insulin to non-diabetic subjects may provoke diabetic responses to glucose tolerance tests. This has been observed in obese (1), undernourished (2, 3), and psychotic (4) patients. In the present experiments, in an attempt to elucidate this phenomenon, the effect of small doses of crystalline and protamine insulin on the respiratory metabolism, as well as on the conventional glucose tolerance test, of normal man has been investigated.

EXPERIMENTAL PLAN AND PROCEDURES

Normal male subjects, maintained on constant diets moderately restricted in carbohydrate, were given small doses of crystalline or protamine insulin for periods of 2 to 6 days. The response of the blood sugar and respiratory quotient to the ingestion of carbohydrate was determined in the morning, before, during, and after the insulin period. The insulin was always given after completion of the day's test.

The authors served as subjects for these experiments. They also made all the analyses except for some of the blood sugar determinations which were made by the technical staff, under the direction of Pauline M. Hald. In addition, they carried on their usual clinical and teaching responsibilities. The subjects avoided strenuous exercise throughout the studies, so that the caloric expenditure must have been fairly constant from day to day; some variation was, of course, unavoidable. Diets were calculated in household measures in the first 3 experiments, by weight in the fourth. In the first 2 experiments, the body weight fell slightly; in the last 2 experiments, a more liberal caloric intake prevented loss of weight.

Fifty grams of glucose in 200 cc. of water, flavored with lemon juice, was used as a test dose, replacing breakfast. The subjects avoided unnecessary activity before coming to the laboratory, where they rested in the reclining position for at least one-half hour before the observations were begun. Oxalated blood samples were taken before, and 90 and/or 120 minutes after, the glucose. In the first 2 experiments, 0.2 cc. of capillary blood was used; in the last two, 2 cc. of venous blood. Protein-free filtrates were prepared within 30 minutes of sampling, by the method of Somogyi (5), for determination of blood sugar in duplicate according to Benedict (6). Duplicate determinations agree within 3 per cent, with rare exceptions. The fasting and post-glucose specimens were analyzed simultaneously, so that comparisons between the two are probably subject to an error no greater than this. Absolute values are less accurate, since, in some instances, the dilute standard solutions were observed to have deteriorated. In control experiments, the blood sugar returned consistently to or below the fasting (or, more properly, post-absorptive) level, 2 hours after the ingestion of the glucose.

Respiratory quotients were determined by standard techniques (7), in the fasting (post-absorptive) state, and 50 and 80 minutes after the glucose. Samples were received in the Tissot apparatus over 10-minute periods; the times refer to the mid-point of these periods. The subjects were all trained in breathing through valves. All gas analyses were made in duplicate, with agreement within 0.03 per cent. Analyses of atmospheric air were run as a further check on technique. In a control experiment, in which water was taken instead of glucose, there was no significant change in either the respiratory quotient or the blood sugar. After at least 30 minutes of rest in the recumbent position, the subject breathed through the valves for 3 minutes before the collection of gas was begun. In each experiment, duplicate basal gas collections were made several times, the first after 30 to 40 minutes and the second after 51 to 66 minutes of rest. The results, presented in Table I, indicate that the respiratory quotient reached a constant level in one-half hour in experiments 1, 2, and 3 but not in experiment 4. The respiratory data of the first experiment, therefore, cannot be interpreted, and will be presented only for those days on which 2 preliminary runs were made.

RESULTS

Before presenting the data for the individual experiments, a preliminary appraisal of the respiratory data is necessary. The increases of

[1] This article represents work done in fulfilment of the thesis requirement for the degree of Doctor of Medicine at Yale University School of Medicine.

TABLE I

Respiratory quotient

Experiment number	Date 1942	Fasting		After glucose		Change from post-absorptive level (× 100) at			
						50 minutes		80 minutes	
		1st run	2nd run	50 minutes	80 minutes	Control	Experimental	Control	Experimental
1	March 30*	0.76	0.75	0.77	0.81		2		6
	April 2	0.75	0.75	0.80	0.82	5		7	
2	March 24	0.76	0.77	0.82	0.84	5		7	
	25	0.78	0.80	0.82	0.84	3		5	
	26*	0.72	0.74	0.74	0.79		1		6
	27*	0.80		0.78	0.80		−2		0
	28*	0.78	0.78	0.84	0.85		6		7
	29*	0.80		0.80	0.84		0		4
	31	0.80		0.85	0.86	5		6	
	April 1	0.81		0.87	0.88	6		7	
	3	0.80		0.84	0.83	4		3	
3	April 9	0.78		0.83	0.85	5		7	
	11	0.81	0.80	0.82	0.87	2		7	
	12	0.78	0.77	0.86	0.86	9		9	
	14*	0.80	0.80	0.82	0.86		2		6
	16*	0.76	0.75	0.75	0.80		0		5
	18*	0.80		0.81	0.82		1		2
	19	0.76		0.82	0.84	6		8	
	20	0.77		0.81	0.82	4		5	
	21	0.77		0.81	0.85	4		8	
	23	0.75		0.81	0.83	6		8	
	28	0.77		0.80	0.79	3		2	
	30*	0.79		0.83	0.85		4		6
	May 1*	0.76		0.77	0.84		1		8
	2*	0.78		0.78	0.86		0		8
4	June 16	0.77	0.75	0.82	0.86	7		11	
	18*	0.79	0.75	0.78	0.82		3		7
	20	0.82	0.78	0.84	0.85	6		7	
					Mean	5.00	1.50	6.69	5.42
					St. Dev.	1.89		2.27	
					t	4.85		1.46	

* Days following insulin administration.

R.Q. over the fasting level at 50 and 80 minutes after glucose ingestion are presented in the last 4 columns of Table I. The experimental observations are those in which insulin has been given during the preceding 24 hours. During 50 minutes after the ingestion of glucose, the mean rise in R.Q. is 0.050 in the control group, 0.015 in the experimental one. Statistical treatment indicates that the difference between these values is highly significant. At 80 minutes, the mean rise is 0.067 in the control group, 0.054 in the experimental one. The difference in these means is probably significant only in that it could have occurred by chance in 1 of 5 trials. It is evident that the rise of R.Q. after glucose arrives at or near a maximum within 80 minutes in all of our experiments, though the rise is probably slightly smaller when insulin has been given on the preceding day. The rise is tardy in the latter instance, however, as demonstrated by the highly significant differences observed at 50 minutes. Since the control and experimental observations approach one another in the 30-minute interval between observations, it is not surprising that there is some overlapping of results at 50 minutes.

The data of the indivdual experiments will be presented graphically, omitting, for the sake of simplicity, the values for R.Q. 80 minutes after glucose. In each of 4 pre-insulin control periods of experiment 1 (Figure 1), the blood sugar 90 minutes after 50 grams of glucose *per os* falls below the fasting level. When 20 units of protamine insulin are given daily, the blood sugar 90 minutes after the glucose is significantly higher than the fasting level. This effect is still present 87 hours after the last injection; a feeding at

bedtime prevents it. Oxidation of the ingested glucose begins less promptly on the morning after the last dose of insulin than it does 3 days later.

In 2 control studies in experiment 2 (Figure 2), the blood sugar 90 minutes after glucose is slightly greater than the post-absorptive level. The difference becomes sharply increased, however, when protamine insulin is given, and remains so 63 hours after the last dose of insulin. The evening and bedtime feedings have no clear-cut effect on the hyperglycemia in this experiment. In 2 control experiments before starting insulin, the R.Q. rises 0.05 and 0.03, respectively, in the 50 minutes after the administration of glucose. When 25 units of protamine insulin are given daily, the corresponding rises are 0.01, 0.02, 0.06, and 0.00. Night feedings result in normal oxidative responses to glucose on the following morning, despite insulin. The oxidative response is also normal 63 hours after the last injection of insulin, though the glucose still provokes excessive hyperglycemia at that time.

In experiments 3 and 4, venous blood was analyzed before and 2 hours after 50 grams of

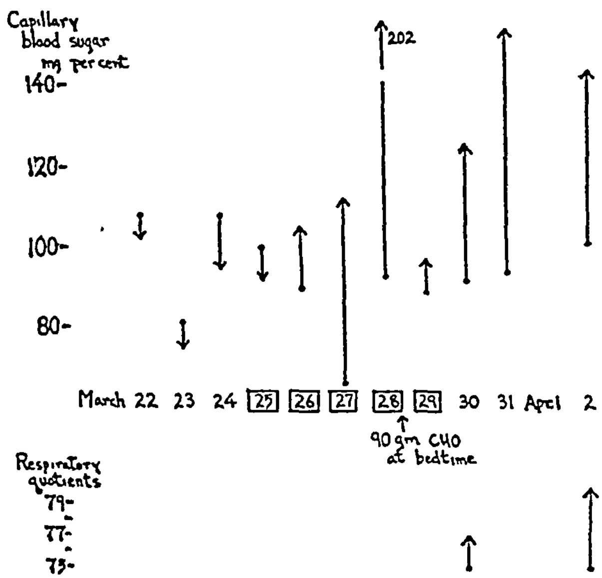

FIG. 1. EXPERIMENT 1

Subject: DeK. Diet P. 80, F. 105, C. 160, including 50 grams glucose at 8 A.M. and 200 cc. of milk at 10:30 A.M. Protamine insulin, U 20, taken in afternoon of days designated by squares about dates. No frank hypoglycemic episodes. Dots represent post-absorptive blood sugar and R.Q. Arrow heads mark values for blood sugar 90 minutes, and R.Q. 50 minutes after 50 grams of glucose *per os.*

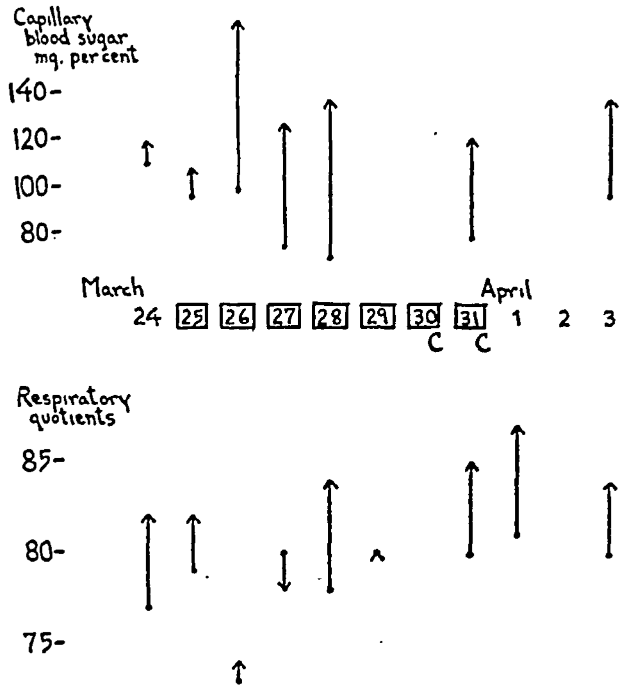

FIG. 2. EXPERIMENT 2

Subject: P. H. L. Diet P. 90, F. 115, C. 220, started on March 21. Protamine insulin, U 25, taken on afternoons of days designated by squares about dates. Mild sweating and restlessness during night of March 25; otherwise no hypoglycemic symptoms. 80 grams of carbohydrate taken in divided doses during the evening of March 30, and 100 grams at bedtime on March 31. Symbols as in Figure 1.

glucose *per os.* This is a conventional test, the normal response to which is a return to or below the original level in 2 hours. Since, in some of our insulin periods, the fasting level is well below that of the control periods, we interpret responses as abnormal only if the 2-hour blood sugar is higher than both the fasting level of the same day and the highest 2-hour level of the control period. With such rigid criteria, even a single abnormal response during the insulin periods becomes significant.

In experiment 3 (Figure 3), 3 control studies were made before each trial of insulin. In none of these did the blood sugar exceed 83 mgm. per cent 2 hours after glucose, and in each instance this was lower than the fasting level. Four of the 8 observations after insulin were abnormal by our criteria, the 2-hour blood sugar being 117 and 112 on 2 occasions. Blood sugars were determined at 90 minutes as well as 120 minutes in

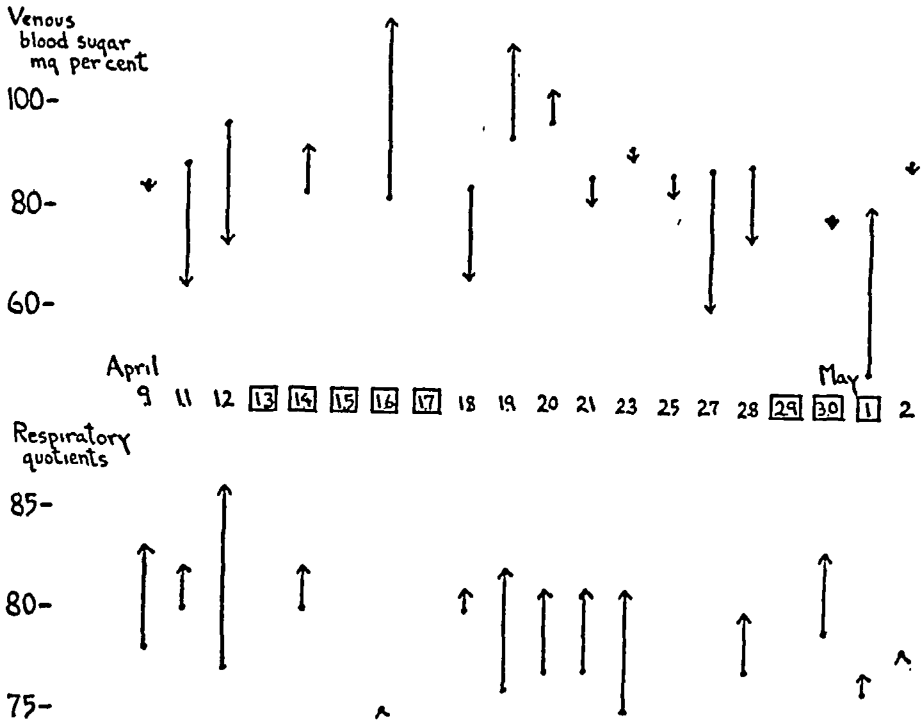

FIG. 3. EXPERIMENT 3

Subject: J. D. R. Diet P. 95, F. 135, C. 175, including P. 8, F. 17, C. 17 in mid-afternoon, started April 7. Protamine insulin, U 15, given each afternoon April 13 to 17 inclusive, and U 22½, 25, and 15 respectively on April 29, 30, and May 1, respectively. One frank mild shock on arriving in laboratory on morning of May 1. Dots represent post-absorptive blood sugars and R.Q.'s. Arrow heads indicate blood sugar 120 minutes, and R.Q. 50 minutes, after 50 grams of glucose *per os*.

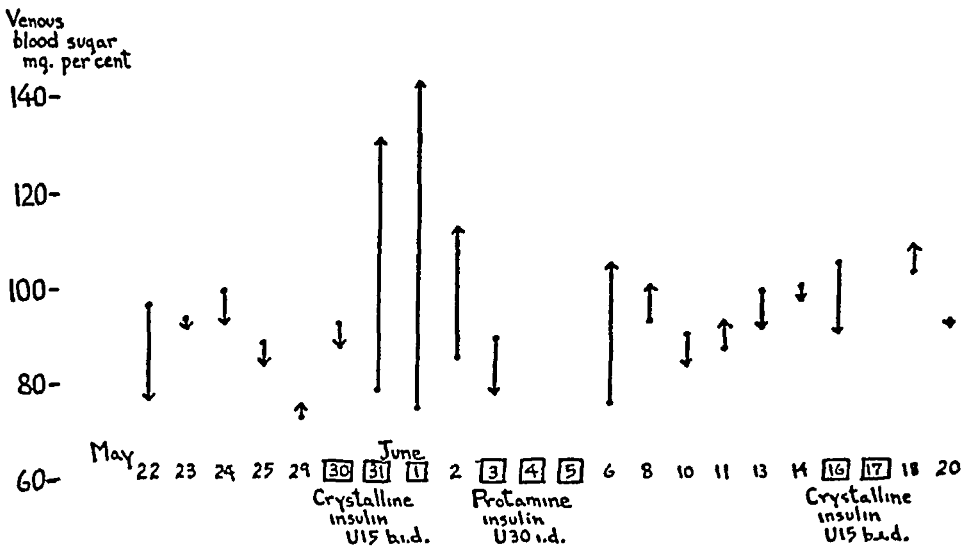

FIG. 4. EXPERIMENT 4

Subject: P. H. L. Diet P. 100, F. 170, C. 230, including 50 grams glucose at 8:00 A.M., P. 13, F. 19, C. 22 at 10:30 A.M., and P. 9, F. 11, C. 22 at 9:00 P.M., started May 19. Crystalline insulin was given at 10:15 A.M. and 5:45 P.M. It provoked mild sweating and tremor just before lunch regularly, and at bedtime occasionally. Protamine insulin, given in the afternoon, induced no hypoglycemic symptoms. Dots and arrow heads as in Figure 3.

this experiment, but since the former parallel the more conventional 2-hour values, they are omitted in the interest of simplicity of presentation. The rise of R.Q. at 50 minutes is 0.05, 0.02, and 0.09 before the first course of insulin; 0.02, 0.00, 0.01 during it; and 0.06, 39 hours after the last dose. With the second course of insulin, the R.Q. fails to rise with normal promptness in 2 or 3 studies. The R.Q. is low during the hypoglycemic episode of May 1, and fails to rise within 50 minutes of the ingestion of the glucose test dose.

In all of 10 control observations in experiment 4 (Figure 4), the 2-hour blood sugar is below 100 mgm. per cent and is lower than the fasting level. This is true in none of the 5 observations of the insulin periods, the 2-hour blood sugar reaching 132 and 144 on 2 occasions. Normal responses return within 36 hours after crystalline insulin, but not within 63 hours after the last dose of protamine insulin. The R.Q. rose only 0.03 in the 50 minutes after glucose administration on June 18, following a trial of crystalline insulin. Control observations on June 16 and 20 show rises of 0.07 and 0.06, respectively (Table I).

DISCUSSION

Since insulin can produce delay in oxidation of carbohydrate in normal subjects on fairly liberal diets, a similar effect in patients with diabetes seems not improbable. This is in keeping with clinical observations that the tolerance of such patients for carbohydrate may be sharply reduced by over-dosage with insulin. Our experiments emphasize the importance of avoiding over-dosage, and of supplying carbohydrate at the times when insulin action is maximal. This is usually in the mid-morning when regular insulin is used before breakfast, and in the evening when it is used before supper. They clearly indicate a need for a bedtime feeding when protamine insulin is used at any time of the day, even though symptoms of hypoglycemia during the night may be lacking.

The mechanism by which insulin reduces tolerance for carbohydrate is not apparent. One possibility is suppression of the insulin-secreting mechanism of the pancreas. There is, indeed, some evidence that the insulin content of the pancreas falls when insulin is given (8). An alternative hypothesis is that insulin, by stimulating oxidation of carbohydrates, reduces that available for formation of liver glycogen (9), establishing a condition comparable to the so-called "starvation diabetes" (10). The two hypotheses are not mutually exclusive since suppression of pancreatic insulin has been produced by restriction of carbohydrate intake (11), as well as by administration of insulin. The occurrence of frank hypoglycemia in experiment 3, when the respiratory quotient is only 0.76, indicating only slight combustion of carbohydrate, suggests that the readily available glycogen stores are depleted, as they are in starvation. The analogy with starvation diabetes is further supported by the observation that the ketones of post-absorptive blood samples were unequivocally elevated on two occasions during the insulin periods. In experiment 1 (March 28), these were 7.1 mgm. per cent, and, in experiment 3 (May 1), 3.2 mgm. per cent, as contrasted with control levels of 2.2 and 1.7 mgm. per cent, respectively, on the same diet without insulin. These determinations were made by Dr. Bernard L. Kartin by the method of Somogyi (12).

Failure of the glucose tolerance curve to return to normal within three days of stopping protamine insulin can only be attributed to continued action of protamine insulin over this period. If protamine insulin had a less durable action, it would be difficult to explain the cumulative effect which makes the time of administration of a daily dose of protamine insulin to diabetic patients immaterial (13). The relative speed with which the oxidative response to glucose reverts to normal after stopping insulin is disturbing, but a similar difference in the speed of recovery of normal oxidative response and of hyperglycemic response to ingested glucose has been observed after starvation (14).

SUMMARY AND CONCLUSIONS

1. In normal man, under controlled conditions, small doses of insulin result in excessive hyperglycemia and delayed rise of respiratory quotient after ingestion of glucose. Both effects are usually evident within less than 24 hours of the start of insulin administration, and both are reversible, the delay in oxidation more promptly than the excessive hyperglycemia. Extra feedings at bedtime prevent loss of tolerance on the following morning.

2. An analogy is drawn between the above effects and "starvation diabetes."

3. The clinical significance of these observations is discussed.

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THE UTILIZATION OF INTRAVENOUSLY INJECTED SALT IN NORMALS AND IN PATIENTS WITH CUSHING'S SYNDROME BEFORE AND AFTER ADMINISTRATION OF DESOXY-CORTICOSTERONE ACETATE[1]

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The following report deals with the utilization of intravenously injected salt, before and after the injection of desoxycorticosterone acetate, in normal individuals and in patients with "Cushing's syndrome."

We observed that, in normal individuals, the intravenous injection of salt following the intramuscular injection of a single dose of desoxycorticosterone acetate resulted in a considerable retention of injected salt, above that seen prior to the injection of the hormone. In contrast to these results, the patients with Cushing's syndrome showed a pronounced sodium chloride diuresis.

It is interesting to note that Kuhlmann and his coworkers (1) and Ragan and his group (2) reported the production of a diabetes insipidus-like condition following the daily intramuscular administration of 20 to 25 mgm. of desoxycorticosterone acetate to normal dogs. These observations were confirmed by Mulinos and his coworkers (3), even though this latter group employed considerably smaller dosages of the hormone.

METHOD

All tests were conducted according to the following standard routine: The patients were permitted no food throughout the period of observation and no fluid after 7 P.M., the night before. At 6 A.M. on the morning of the studies, the patient was asked to void and the urine discarded. He was then given 500 cc. of water to drink at one time, and all the urine voided was collected over a 3-hour period to 9 A.M., carefully labeled and set aside. At 9 A.M., 200 cc. of 5 per cent saline (10 grams of salt) were injected intravenously and the urine voided during the next 3 hours, to 12 M., was similarly collected and labeled. Two days later, the same procedure was repeated, except that the patient was injected the previous evening at 10 P.M. with 10 mgm. of desoxycorticosterone acetate, intramuscularly. The urine volume of each period was carefully measured and sodium and chloride determinations were made on each specimen. Sodium was determined according to the method of Butler and Tuthill (4), and chlorides according to the method of Van Slyke (5). The total urinary sodium and chloride ions excreted during the 6 A.M. to 9 A.M. period were subtracted from those excreted between 9 A.M. and 12 M. (period after the intravenous injection). The difference represents the excess over the basal excretion of these ions. This figure in milliequivalents, divided by 171 (*i.e.* milliequivalents in 10 grams of sodium chloride), yields the fraction of the injected ions excreted (Na_C and Cl_C). The comparison of the results obtained during the control period and that after the injection of desoxycorticosterone acetate showed the percentage of increased retention resulting from the use of the hormone.

RESULTS

In Table I are presented the results obtained in the normal individuals and in the patients with Cushing's syndrome. It will be seen that, with the exception of one instance, all the normals showed a considerable increase in the percentage of injected salt retained after the injection of desoxycorticosterone. The patients with Cushing's syndrome, on the other hand, retained a much smaller percentage of the injected salt after administration of the hormone than they did during the corresponding control studies. In general, the urinary excretion of the sodium and chloride ions ran parallel to one another, both during the control studies and after the injection of the hormone.

DISCUSSION

The following tentative explanation of the contrasting results obtained in normal individuals and in patients with Cushing's syndrome is of-

[1] Aided by a grant from the Ciba Pharmaceutical Company, Summit, N. J. The desoxycorticosterone acetate (Percorten) was generously supplied by Mr. Robert C. Mautner of the Ciba Pharmaceutical Company.

[2] Lieutenant, M. C., A. U. S.

[3] Moses Heinemann Fellow.

TABLE I

	Control				After injection of 10 mgm. of desoxycorticosterone acetate				Excretion			
	Urine volume		Injected Na excreted Na_C	Injected Cl excreted Cl_C	Urine volume		Injected Na excreted Na_D	Injected Cl excreted Cl_D	Decrease		Increase	
Normals	6 to 9 A.M.	9 to 12 A.M.			6 to 9 A.M.	9 to 12 A.M.			$100\frac{(Na_C - Na_D)}{Na_C}$	$100\frac{(Cl_C - Cl_D)}{Cl_C}$	$100\frac{(Na_D - Na_C)}{Na_C}$	$100\frac{(Cl_D - Cl_C)}{Cl_C}$
	cc.	*cc.*	*per cent*	*per cent*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*		*per cent*	
S. B.	245	190	4.9	12.7	304	125	2.7	8.8	44.8	30.6		
M. L.	778	333	24.5	32.3	662	241	−1.2	−2.6	>100	>100		
A. B.	170	170	12.6	10.7	192	132	4.8	2.4	61.9	68.8		
B. C.	503	360	24.1	27.4	510	254	15.8	16.1	34.4	41.2		
H. R.	710	270	19.3	21.8	500	260	13.8	19.3	28.5	11.4		
H. S.	565	230	19.2	25.1	420	250	−20.6	−6.0	>100	>100		
R. O.	250	152	3.7	7.3	335	150	5.6	13.8			51.3	89.0
R. M.	420	165	17.6	17.2	248	108	7.5	12.3	57.3	28.4		
W.	235	284	10.5	15.2	316	126	1.5	−1.5	85.7	>100		
Z.	252	108	8.6	5.7	148	88	3.4	4.1	60.4	28.0		
Y. F.	26	83	0.2	1.0	27	34	0.1	0.1	36.8	88.5		
D. K.	970	380	29.8	34.5	770	300	26.5	30.8	11.0	10.7		
Cushing's syndrome												
E. S.	245	190	26.7	32.0	297	950	70.3	59.8			163.2	87.0
A. P.	378	336	8.9	9.1	380	418	19.8	19.5			122.4	114.2
A. P.	63	600	39.5	39.1	265	900	59.7	60.0			51.1	53.4
E. G.	650	250	11.8	12.2	418	238	17.3	19.0			46.6	55.7
T. S.	255	190	10.8	14.8	274	365	39.6	36.1			280.7	143.8
J. S.	342	130	5.9	5.9	315	124	7.8	9.1			32.2	54.2

The percentages of injected Na and Cl excreted (Na_C and Cl_C) were computed as follows: The total Na and Cl output for the 6 to 9 A.M. period was subtracted from that of the 9 to 12 period. The resulting amount is expressed in milliequivalents, divided by 171 (equals number of milliequivalents Na and Cl contained in 10 grams NaCl), and multiplied by 100 to yield percentages. The same procedure was followed after the injection of hormone, yielding the percentage Na_D and Cl_D.

fered. It is interesting to note the infrequency with which edema occurs in the normal animal following treatment with desoxycorticosterone acetate, as contrasted to the bilaterally adrenalectomized one so treated (*2*). We have noted a similar phenomenon in the treatment of patients with Addison's disease and in the experimental use of the hormone in individuals who have no evidence of adrenal cortical disease. The former are very much more prone to the development of edema following the use of the hormone. It would appear that some compensatory mechanism operates in animals and patients with intact adrenals that delimits the salt-retaining effect of the desoxycorticosterone. This is evidenced not only by the above findings, but also by the fact that patients with adrenal cortical tumors do not develop edema. In the presence of such tumors, one might expect that the increase in mass of adrenal cortical tissue, theoretically resulting in increased formation of salt-retaining hormone, would cause progressive edema. The fact that such edema does not occur would suggest several possibilities: (*1*) That the salt-retaining hormone is not formed in excess in these instances; (*2*) that excessive salt-retaining hormone formed is rapidly converted into another substance lacking salt-retaining effects; or (*3*) that the formation of excessive amounts of salt-retaining hormone stimulates the production of diuretic hormones, either in the adrenal itself or in the anterior lobe of the hypophysis. The results obtained in our studies would suggest that either or both of the last two possibilities operates. The conversion of the desoxycorticosterone into a non-salt-retaining hormone would at least seem theoretically possible in view of the close chemical similarity between desoxycorticosterone and other adrenal steroid hormones which have no salt-retaining effect, such as Corticosterone, or which actually

expedite sodium and chloride excretion, such as 17-Hydroxycorticosterone.

CASE REPORTS OF PATIENTS WITH CUSHING'S SYNDROME

E. S. Female, age 37. This patient was well until 2½ years ago, at which time she noticed a slight but definite decrease in visual acuity. One and one-half years ago, she developed large ecchymotic areas over the lower extremities, which have recurred at frequent intervals since. Six months ago, she noticed a change in the appearance of her face. Her face became round, puffy, plethoric, with coarsening of the features and marked hirsutism. During this period of time, she developed amenorrhea. On physical examination, she was found to be very obese. She had many purplish striae over the abdomen and large ecchymotic areas over the lower extremities. Pelvic examination failed to reveal any adnexal masses. The blood pressure was 154/114. Hemoglobin was 105 per cent; red blood cells, 5.4 million; white blood cells and differential were normal. The oral glucose tolerance test, employing 1.75 grams of glucose per kgm. of body weight, yielded the following results: Control, 70 mgm. per cent; ½ hour, 150; 1 hour, 250; 2 hours, 175; 3 hours, 210; 4 hours, 110; and 5 hours, 65 mgm. per cent. The serum cholesterol was 230, calcium was 8.8, and inorganic phosphorous 2.9 mgm. per cent; phosphatase was 9.4 K–A units, and chlorides, 115 m.eq. per L. Urine showed frequent traces of sugar. X-ray studies showed a normal sella, marked osteoporosis of the entire spine, and an old fracture of the left fifth rib in the anterior axillary line. Peri-renal insufflation revealed a mass on the left side, and, on operation, an adrenal cortical tumor the size of a small plum was removed.

A. P. Male, age 27. In 1941, the patient noticed a rapid gain in weight, swelling and redness of the face, thinning of the hair, polyuria, polydypsia, diminution in the size of the penis, and loss of libido. On physical examination, he was found to be an obese man with florid, buffalo-like facies, and marked purplish axillary and abdominal striae. The blood pressure was 178/104. The blood hemoglobin was 91 per cent with 4.4 million red blood cells per c.mm. The white blood count and differential were normal. The urine showed a 4-plus sugar. The blood urea N was 11 mgm. per cent and sugar 200. The serum cholesterol was 440, esterified cholesterol 225, calcium 10.1, inorganic phosphorous 2.7 mgm. per cent, and chlorides, 100 m.eq. per L. The blood phosphatase was 19 K–A units. The glucose tolerance curve was typically diabetic in character with a 2-hour rise to 410 mgm. per cent. The basal metabolic rate was —31 per cent. The visual fields showed slight temporal constriction of the peripheral fields. X-rays of the skull and long bones were normal, but the lumbosacral spine showed moderate generalized osteoporosis with compression of the vertebral bodies. Peri-renal insufflation failed to reveal any adrenal masses.

E. G. Female, age 33. Ten months before admission to the hospital the patient developed amenorrhea which has persisted to date. During this period of time, she developed marked facial hirsutism, swelling, redness and roundness of the face, an acneform rash over the back, and gained 12 pounds in weight. The physical examination revealed a short, obese, plethoric-looking woman with a marked kyphosis. There were deep reddish striae over the left flank, and the fingers and toes showed some acrocyanosis. The pelvic examination was negative. The blood pressure was 160/100; hemoglobin, 100 per cent; red blood cells, 5.2 millions per c.mm., and the urine was negative for sugar and Bence-Jones protein. A glucose tolerance test yielded the following results: Control, 95 mgm. per cent; ½ hour, 185; 1 hour, 200; 2 hours, 180; 3 hours, 160 mgm. per cent. The serum cholesterol was 370 mgm. per cent, the serum calcium was 10.0, inorganic phosphorus, 3.5 mgm. per cent, and chlorides, 120 m.eq. per L. The blood urea N was 21 mgm. per cent. The blood phosphatase was 20 K–A units. The basal metabolic rate was —16 per cent. Roentgenologic studies showed slight generalized decalcification of the skull and long bones. The sella turcica was normal. There was extensive decalcification of the entire spine with compression fractures of the 6th, 8th, and 9th dorsal, and 1st lumbar vertebrae. There was, in addition, a transverse fracture through the left 7th rib in its axillary portion. Peri-renal insufflation failed to reveal any adrenal masses.

T. S. Female, age 18. During a period of 4 years prior to admission to the hospital, the patient developed marked facial and body hirsutism. One year before admission to the hospital, amenorrhea occurred, which has persisted. During the course of this past year there was a marked gain in weight. Physical examination revealed a very obese girl with a round plethoric face. There was a diffuse acneform eruption over the face and back. Many purplish striae were seen over the upper arms, breasts, and abdomen. Pelvic examination was negative. The blood pressure was 146/105. Urine was negative for sugar, and the basal metabolic rate was —19 per cent. The blood hemoglobin was 78 per cent, and the red blood cell count was 4.5 millions per c.mm. The glucose tolerance curve was as follows: Control, 75 mgm. per cent; ½ hour, 115; 1 hour, 170; 2 hours, 205; and 3 hours, 150 mgm. per cent. The serum calcium was 10.1, and the inorganic phosphorus, 2.5 mgm. per cent. The total serum proteins were 6.6 grams per cent. X-ray of the sella turcica was normal. Both adrenals were explored, but no evidence of tumor masses was found.

J. S. Female, age 34. Suspected early Cushing's syndrome. One year prior to admission to the hospital, this patient noticed the gradual appearance of considerable facial hirsutism and a rapid and rather marked gain in weight. Her physician told her that she had developed hypertension. There were no irregularities in her menses, and no loss in sexual desire. On physical examination, she was found to be an obese woman with a round plethoric face. She had many non-violaceous striae over the lower abdomen and flanks. Pelvic examination was normal. The blood pressure was 140/100. The urine was negative, and the basal metabolic rate was —19 per cent.

few curves were presented showing the speed of removal of nitrogen from normal and emphysematous lungs in the course of oxygen breathing. The gross differences were apparent, but these curves could not be analyzed quantitatively because periodic alveolar sampling modified the normal breathing pattern.

More recently Engelhardt (11) measured the expired nitrogen in successive breaths of pure oxygen. Assuming a constant dead space of 150 ml., he found less nitrogen expired than he predicted from the volumes concerned, and from this, he postulated an unventilated lung portion or "verweilluft" which amounted to 76 per cent in normal relatively shallow breathing and 23 per cent in very deep breathing. However, in our experience, the value for the upper pulmonary dead space is rarely as small as 150 ml., especially if the dead space of the mouthpiece is included. Recalculating his data, using a value of 175 or 200 ml. for the dead space, one finds that the "verweilluft" becomes 20 per cent or less on shallow breathing as well as on deep breathing. This demonstrates the importance of an accurate estimate of the dead space in this type of calculation when the tidal air is small.

In the present experimental program, we have studied the speed of nitrogen removal from the lungs by means of the inhalation of oxygen during regular quiet breathing. If the basic quantitative factors involved are accurately known, *i.e.*, the size of the pulmonary air space, the effective tidal air (tidal air minus pulmonary dead space), and the number of respirations per minute, and *if perfect mixing of each tidal breath throughout the total pulmonary air space is assumed*, then the amount of nitrogen remaining in this air space, at the end of any given number of breaths, can be calculated. Conversely, in actual experiments in which the subject breathes oxygen, the degree of *imperfectness* of mixture can be determined by the extent by which the nitrogen fails to reach the low value predicted by the theoretical calculation.

To derive the formula for the predicted rate of nitrogen removal, let us consider the situation following one normal breath of pure oxygen. The volume of gas in the lungs at the start of this breath is the functional residual air (R), which has a nitrogen concentration of approximately 80 per cent (a). It is assumed here and in later considerations that the normal breathing is deep enough to wash out the upper pulmonary dead space at each expiration so that, at the start, this dead space is filled with alveolar air of the previous breath. At the end of inspiration, the dead space is filled with oxygen and all the nitrogen is in the lungs in a volume equal to R plus the effective portion of the tidal air (T′).

Lung nitrogen concentration at 1st breath,

$$x_1 = a\frac{R}{R + T'}.$$

If the oxygen is not pure but contains a measurable nitrogen concentration (b), then

$$x_1 = \frac{aR}{R+T'} + \frac{bT'}{R+T'}, \quad \text{or} \quad x_1 = (a-b)\frac{R}{R+T'} + b$$

For simplicity let us consider the expression "$\frac{R}{R + T'}$" as a ratio (r) which represents the dilution rate per breath, in the sense that when r is small, lung nitrogen decreases rapidly on breathing oxygen. Then, following similar considerations:

Lung N_2 concentration at second breath $= x_2 = (a - b)r^2 + b$.

Lung N_2 concentration at third breath $= x_3 = (a - b)r^3 + b$.

Lung N_2 concentration at *nth* breath $= x_n = (a - b)r^n + b$.

For very short periods of only a few breaths, the nitrogen released from the blood (hereafter referred to as "nitrogen excretion") is small and may be disregarded. It must be estimated for experiments when the time is longer and when the gradient between blood and lung nitrogen tensions has been raised sufficiently. The total nitrogen excretion in 7 minutes has been found to average 220 ml. (12). Although Behnke *et al.* (13) have established an exponential type of curve for the rate of excretion over long periods of time, the rate may be considered nearly constant for the first few minutes $\left(i.e., \frac{220}{7} \text{ ml. per minute}\right)$. The figure desired for our calculation is the rise in lung nitrogen concentration caused by excretion in the time of one breath.

Designating this figure as "c,"

$$c = \frac{N_2 \text{ excretion per minute}}{(\text{Respiration rate per minute}) \times (R)}.$$

Introducing this value into the simpler formulae for predicting the lung nitrogen concentration, one obtains the following series of corrected formulae:

$$x_1 = (a - b)r + b + c,$$
$$x_2 = (a - b)r^2 + b + c(1 + r),$$
$$x_3 = (a - b)r^3 + b + c(1 + r + r^2),$$
$$x_n = (a - b)r^n + b + c\frac{(1 - r^n)}{1 - r}. \quad \text{Formula } (A)$$

The solid lines in Figure 2 illustrate the form of curve obtained from this formula, and thus describe quantitatively the progressive fall in intrapulmonary nitrogen, as the breathing of pure oxygen continues,—assuming that intrapulmonary mixing is perfect.

As "n" becomes very great, "x_n" approaches $b + c\frac{1}{1 - r}$, which is a mathematical expression for the lowest lung nitrogen concentration obtainable in the first few minutes. In actual experiments, it usually checks the measured value.

METHODS

The apparatus was similar to that used in the open circuit method of measuring residual air, described in a previous paper (10) (Figure 1). An addition to the previous apparatus was a moving drum (D) and a recording pen, attached to the Tissot counterweight. The distinctive feature is a special valve (V_1) adjacent to the mouthpiece. With this, the subject's breathing may be turned into either (*1*) a main circuit where a bag kept full of oxygen furnishes the inlet gas and the expired gases are collected in a Tissot gasometer (T) whose bell dead space has been determined; or (*2*) a side circuit for breathing room air before and after the period of the experiment and for alveolar sampling. For the latter, a second valve (V_2) closes the inspiratory side of this circuit.

As a preliminary to each experimental period, on each subject, the upper pulmonary dead space was estimated from measurements of tidal air volume, and of the expired and alveolar gas concentration during a 3-minute period, with the subject quietly breathing room air. The dead space was calculated from Bohr's formula (14) both in terms of carbon dioxide values as

$$\text{D.S.} = \text{tidal air} \times \frac{\text{alv. } CO_2\% - \text{exp. } CO_2\%}{\text{alv. } CO_2\% - 0.03}$$

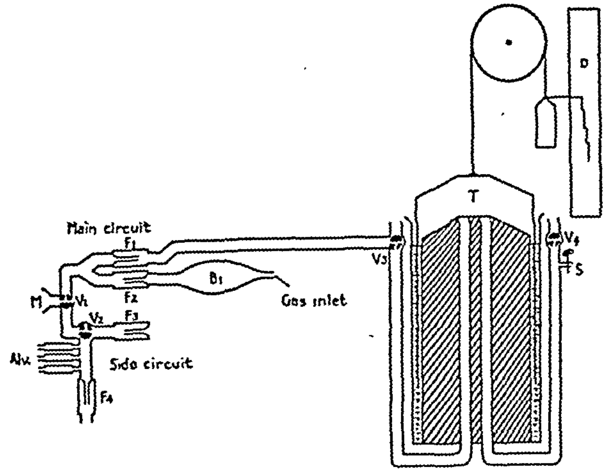

Fig. 1. Diagram of Apparatus

M = mouthpiece; V_1 = valve for turning from one circuit to the other; V_2 = shut-off valve for alveolar sampling; V_3 and V_4 = gasometer control valves; "Alv." = alveolar sampling tubes; F_1, F_2, F_3, F_4 = flutter valves; B_1 = rubber bag for inlet gas; T = Tissot gasometer; s = sampling tube for gasometer; D = recording drum.

and in terms of oxygen as

$$\text{D.S.} = \text{tidal air} \times \frac{\text{exp. } O_2\% - \text{alv. } O_2\%}{20.93 - \text{alv. } O_2\%}.$$

We have used the latter (usually larger) value throughout, because it gives a slower predicted rate of mixing and is the value less liable to give a false conclusion of incomplete intrapulmonary mixing. For the same reason, we have neglected the small correction in the alveolar air analysis which might be made for the gas exchange during the time of sampling.

To determine a point on the *actual* mixing curve, the subject, previously breathing room air, was allowed to begin breathing oxygen, exactly at the end of a normal expiration. He was instructed to avoid sighing during the experiment. After the desired number of breaths of oxygen, the last normal expiration was allowed to go into the gasometer; then a forced expiration was made into the alveolar sampling arm, with its evacuated gas sampling tubes attached. (Before the experiment, the entire main circuit and gasometer had been thoroughly flushed out with oxygen.) Immediately after the alveolar sampling, the nitrogen still remaining in the tubing of the main circuit was washed into the bell, the total volume read, and a sample of the mixed gases taken from the gasometer. From these data, the total volume of expired nitrogen was directly calculated. The tracing of expirations was measured for rate and average depth of breathing. Experiments showing markedly irregular breathing were discarded.

On each experimental day, this procedure was repeated 4 to 12 times, the number of breaths (n value) being increased in each successive O_2 breathing period, so as to give significant points on the entire mixing curve. An interval of at least 15 minutes of rest between O_2 breathing periods

was allowed, so that at least the bulk of the nitrogen released from the blood could be reabsorbed. The last experiment of the day was used to determine the value of the functional residual air (R). In this case, the time of breathing pure oxygen continued beyond the washing-out period, usually to 7 minutes.

Data from the above procedures afford two separate estimates of the pulmonary nitrogen concentration after various numbers of breaths of oxygen: (*1*) The alveolar specimens represent direct attempts at such an estimate. (*2*) The values for expired nitrogen subtracted from the total lung nitrogen (measured in the determination of R) give an estimate which is independent of the subjects' ability to expire a uniform alveolar sample. The calculation for this second estimate, expressed mathematically, using the same symbols as in previous formulae, becomes:

$$x_nR = aR - (N_2 \text{ expired in n breaths}) + (N_2 \text{ excreted in n breaths}).$$

This measurement is accurate only in the first one-half or two-thirds of the mixing curve when the difference between the two volumes of nitrogen is relatively great and the factor of nitrogen excretion is minimal.

In experiments where both methods of calculation are possible, the comparison of the two values furnishes another test of the uniformity of the alveolar air. If they differ significantly, it may be assumed that the alveolar sample is not representative of the average gas concentration existing at the moment of sampling. Divergence might occur with the rapid changes of intrapulmonary gas contents during oxygen breathing, even though, during breathing of ordinary air, the alveolar samples are considered satisfactory, judging by the criterion that the CO_2 pressure approximates that of the arterial blood. Actually, all the subjects studied in these experiments could deliver good alveolar samples according to this criterion.

RESULTS

The subjects consisted of 18 normal adults (15 male and 3 female), and 5 patients with severe pulmonary emphysema. The normal subjects included physicians, medical students, and ambulant patients, suffering from non-respiratory disease. The entire group ranged in age from 21 to 65 years.

Figure 2 presents the results of two typical experiments, in which are plotted the lung nitrogen concentrations as ordinates against the number of breaths ("n") as abscissae. The "n" scale is presented logarithmically for convenience because the points are taken closer together in the beginning of the curve than in the end. In each experiment, the "r" value (r = dilution rate per breath) varied somewhat from one breathing period to another because of the unavoidable variations in average tidal air. The complete mathematical curves (cf. formula, above) are drawn for the largest and the smallest values of "r" as found in each experiment, to show the shape of the curves and the range of possible values for the experiment in question. From the data of each period of oxygen breathing, three points are presented on the chart at the "n" value of that breathing period:

(*1*) The open circles (o) represent the *theoretical* nitrogen concentrations existing after n breaths, assuming perfect mixing, *i.e.*, by the use of formula (*A*); (*2*) the solid circles (•) represent the actual *experimental* nitrogen concentrations after n breaths, using the measured concentration of nitrogen in alveolar air for this factor; (*3*) the solid triangles (▲) also represent actual experimental nitrogen concentrations, but obtained, in this case, by subtracting the total expired nitrogen from the initial total nitrogen in the pulmonary air space.

It will be seen that, on subject 4, the three points show only slight divergence, which may be attributed to experimental errors in the method. In other words, in this subject, the lungs appeared to behave as a perfect mixing chamber. Assuming the dead space from Bohr's calculation, each effective tidal air volume mixed uniformly with the total pulmonary (*i.e.*, functional residual) air volume. Also, the usual alveolar sampling method gave a value equal to the average concentration in the lungs at that moment.

In the case of the typical experiment on subject 6, a different result will be seen. This subject was a man of 65 whose heart and lungs were normal as far as could be determined. Yet as will be seen: (*a*) The "calculated" lung nitrogen values (definition (*3*) above) were regularly higher than the predicted value (definition (*1*) above). (*b*) The "alveolar" samples (definition (*2*) above), being lower than the "calculated" values and in three instances even lower than the "predicted" values, apparently gave an erroneous measure of average lung nitrogen concentration. The situation may be pictured as follows: each breath of oxygen mixed completely with only a portion of the alveolar air; in the remainder, the nitrogen was washed out only slowly. At the end of the breathing period, there were presumably various concentrations of nitro-

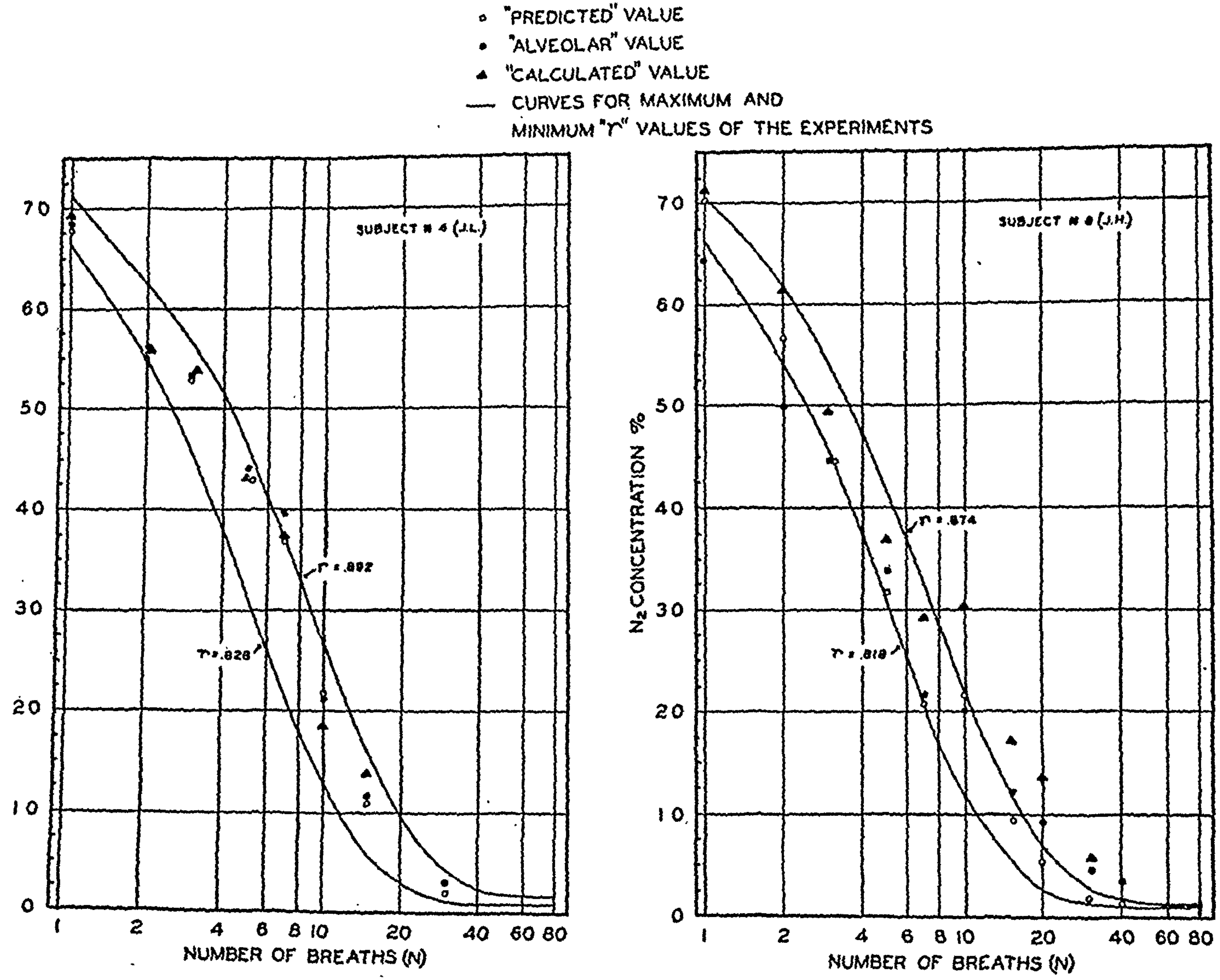

FIG. 2. SAMPLE EXPERIMENTS ON 2 SUBJECTS

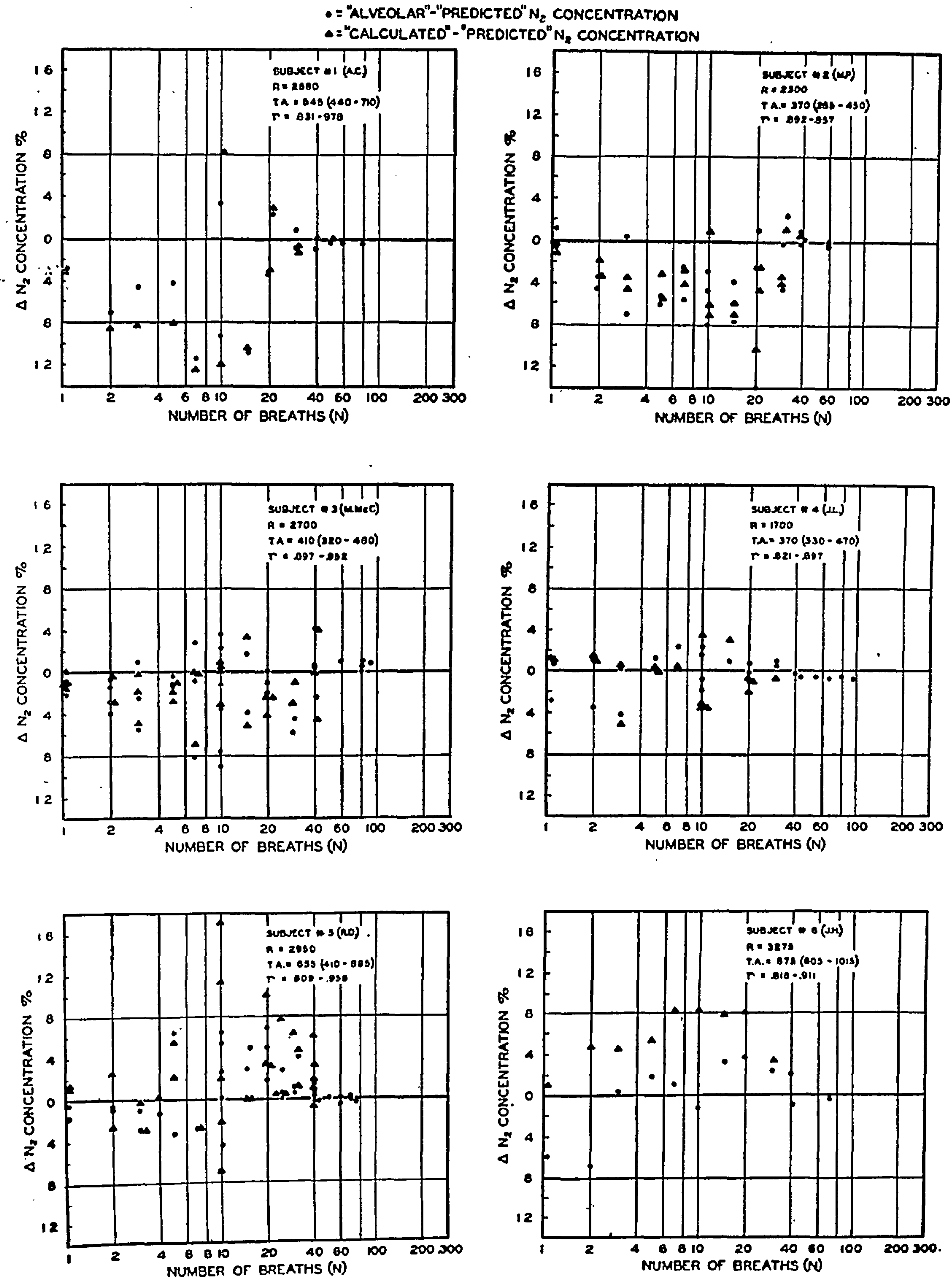

Fig. 3. Summary of Data on 6 Normal Subjects

gen in different alveoli. The alveolar sample therefore came chiefly from the well-ventilated portions and so was below average in nitrogen concentration.

With these typical experiments as a background, we may now turn to a survey of all our data. Figure 3 presents the results on 6 normal subjects, which were those most thoroughly studied. Data on each of the other 12 normal subjects corresponded closely to those of one or another of the 6 presented in detail. Figure 4 presents, similarly, the results on 4 subjects with pulmonary emphysema.

The graphical scheme is as follows: the abscissae represent the number of breaths (n) on a logarithmic scale as in Figure 2. The ordinates, labelled "ΔN_2 Concentration," represent differences between the predicted (theoretical perfect mixing) values for each experiment (cf. derivation of formula in Introduction) and the values actually measured. The symbols remain the same as in Figure 2: the circles for alveolar samples directly analyzed, the triangles for the values for pulmonary concentration of nitrogen calculated from the expired nitrogen (cf. derivation under Methods).

This method of presentation offers several advantages over the more complete picture as in Figure 2: (*1*) It eliminates one of the points plotted in each experiment, *i.e.*, the predicted value, which may be considered to be set on the base-line. (*2*) The plotting of divergences from predicted values reduces the range of the vertical scale to a more convenient size. (*3*) The arithmetical divergences are themselves the figures of greatest interest since they directly indicate the adequacy or inadequacy of intrapulmonary mixing.

Looking at the charts on individual subjects, it will be seen on subjects 1 and 2 that the preponderance of points fall below the base-line of predicted values. No significant divergence between the circles and triangles can be noted. In other words, the nitrogen actually measured as remaining in the lungs after n breaths is less than the predicted (perfect mixing) value. Such a situation can be interpreted only as due to errors in one or more of the assumptions used in the formula for predicted values. It was mentioned that we used the larger value for upper pulmonary dead space, derived from oxygen values by Bohr's formula, and that we neglected alveolar corrections for gas exchange during the time of sampling. These assumptions were intentionally made to give the highest possible predicted values. Thus a deviation above the base-line is doubly significant; one slightly below the base-line, of little or no significance.

Charts on subjects 3 and 4 show a much closer approximation of the measured to the predicted values. This is representative of the majority of the normal subjects studied.

In the case of subject 5, there is a rather wide scatter of points but clearly there is a preponderance of points above the base-line, especially at $n = 10$ and $n = 20$. As discussed above, deviations in this direction are of real significance and probably indicate the effect of unequal ventilation of the different lung portions and thus imperfect mixture. The reason for variations in response in different normal subjects is one of the questions which will be discussed in a later section.

The sixth chart is taken from data on J. H., the eldest subject in the group (age 65). Aside from a mild chronic nephritis, without hypertension or heart disease, he was in good physical condition. It will be seen for the first time among the charts discussed that the values for "calculated" lung nitrogen (▲) lie regularly much higher than the "alveolar" values (●). In addition, the former values are well above the base-line. Thus, in this case, both of the criteria for incomplete alveolar mixing are satisfied. We may visualize that nitrogen persisted in some regions of the lung after it had been nearly washed out of other portions and that some of this nitrogen failed to be expired, even during the forced breath of alveolar sampling. Such a situation was demonstrated in 2 other normal subjects in the group, both over 50 years of age. The question may be raised whether the lungs of these middle-aged or elderly men were entirely normal. At present, we can only say that this picture forms a transition from the normal to that seen in clinically abnormal subjects.

The last 4 charts (Figure 4) on abnormal subjects are presented in order of increasing abnormality. In all, there is a wide divergence between alveolar (●) and calculated (▲) values

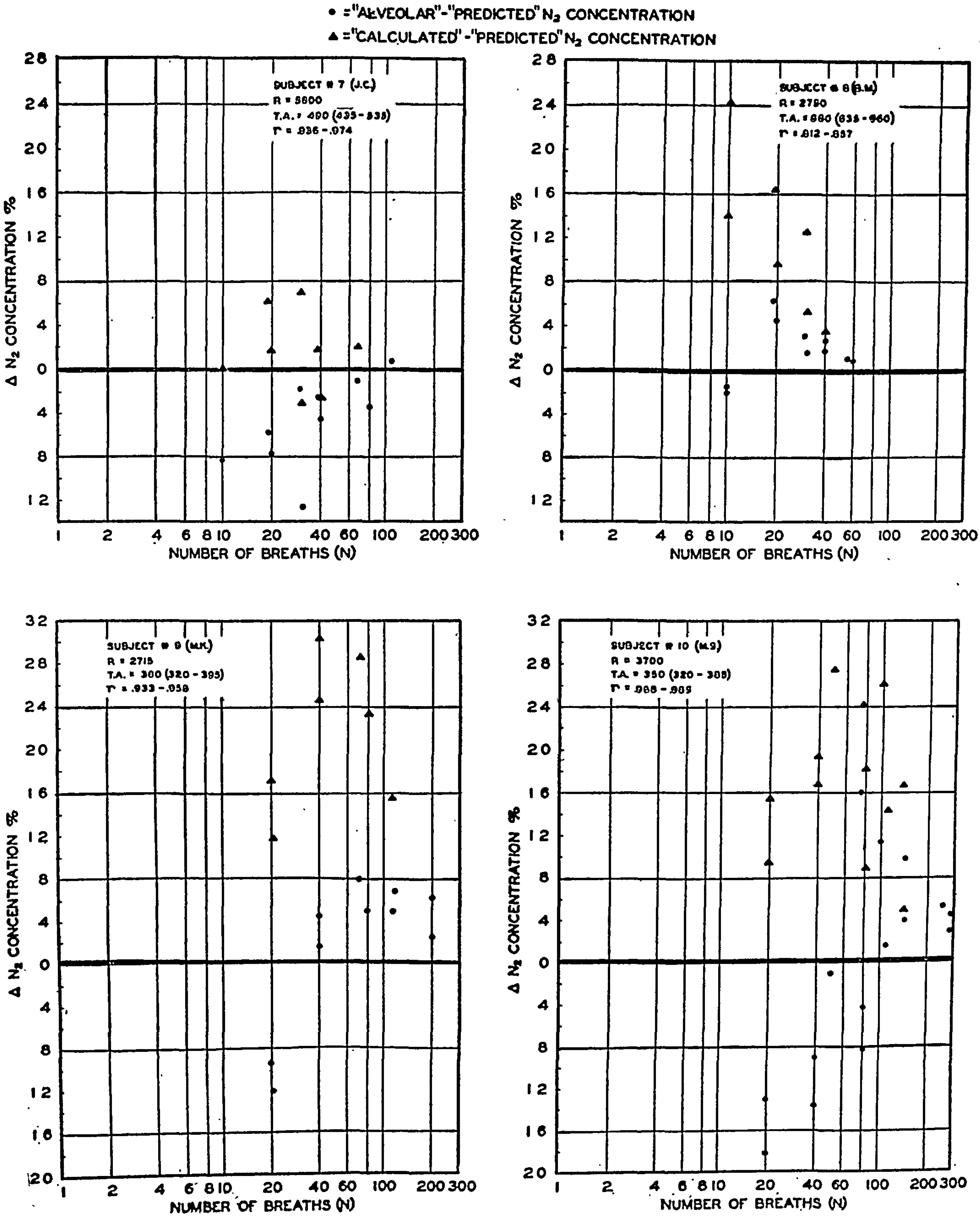

FIG. 4. SUMMARY OF DATA ON 4 SUBJECTS WITH SEVERE PULMONARY EMPHYSEMA

during the first part of the mixing curve. In the last 3 subjects, the calculated values lie high above the predicted values, higher by far than any points in the normal subjects. In other words, much more nitrogen still remains in the lungs of these subjects after n breaths of oxygen, than that which would have remained if intrapulmonary mixing had been perfect.

In subject 7, the calculated values lie above the line but somewhat less so than the last normal subject (Number 6). Since subject 7 was suffering from severe respiratory disability, it is of interest to discover possible differences between him and the other 3 on Figure 4.

Unlike the others, he did not show significant arterial oxygen unsaturation, although he suffered from severe dyspnea on the slightest exertion. However, his functional residual air volume was by far the largest of the 4—over 5,000 ml. as compared with 2,500, 2,700, and 3,700 in the other 3. These facts, together with the data on the speed of mixing, almost surely distinguish two important types of pulmonary disability, about which we have previously had no distinguishing test. In fact, principal emphasis has been placed on the increase in residual air, together with the fall in vital capacity, to explain the disability in pulmonary emphysema. From a critical analysis of usual residual air measurements in a previous paper (3), it was concluded that frequently, in such cases, the measurements gave falsely high residual air values due to the factor of imperfect intrapulmonary mixture. At that time, the latter factor could not be accurately measured.

Now we may conclude that subject 7 suffered his chief disability from the disturbances in lung size and gross mechanics, and that the other 3 subjects, with less of this type of disability, had in addition a marked degree of retarded and inadequate intrapulmonary mixing. Presumably the latter defect is due to narrowing or distortion of the bronchiolar structure.

DISCUSSION

In the data presented above, certain qualitative facts stand out: (*1*) Some normal subjects appear to have nearly perfect mixing of each inhaled breath through the alveolar spaces. (*2*) Other apparently normal subjects have small to moderate degrees of imperfect mixing. (*3*) Three of 4 cases of emphysema showed a striking inadequacy of mixing of inhaled air evenly through the lungs. One emphysematous subject, with a very large functional residual air, had defective pulmonary emptying on this account, but the distribution of inhaled air throughout this large lung volume was relatively good.

Discussion of errors

One limitation on accuracy in these and previously published experiments is the difficulty in accurately estimating the upper pulmonary dead space. Some workers, especially Haldane (15 to 18), found that the dead space increased markedly with deeper breaths; others, chiefly Krogh and Lindhard (19 to 21), found a constant dead space. Schoedel (22) would seem to have reconciled the differences by more careful consideration of the changes in concentration during sampling, thereby establishing that the dead space is nearly constant, except in pathologically shallow breathing.

A few direct experiments on this subject are presented in Table I. Here, the tidal air was

TABLE I

Values of the dead space of the upper respiratory spaces, determined at different depths of quiet breathing

Subject	Tidal air	Dead space	
		From CO_2 values	From O_2 values
	ml.	*ml.*	*ml.*
J. H.	670	293	351
	815	274	286
	900	304	372
	980	308	358
M. McC.	345	176	194
	355	172	173
	390	180	203
	395	193	216
	425	193	213
M. P.	315	167	180
	385	174	168
	460	184	183
	475	173	177
	480	171	211
J. L.	285	147	146
	305	124	132
	345	147	158
	380	151	164
	385	150	190
	600	161	179

varied at rest, keeping the total ventilation constant by reducing the respiratory rate. Because of the difficulties of such voluntary regulation, the results embody more errors than would be ideal, yet it is definite that the dead space increases only slightly, if at all, with increased depth of breathing.

With this evidence as justification, we have assumed a constant dead space in our "predicted values" when the tidal air differed from the volume used in Bohr's formula. However, in actual experiments, the tidal air varied both up and down from the standard value, so that the *mean error from such an assumption should not* be great. This may explain in part the scatter of points found in some normal subjects.

Another cause of scatter is undoubtedly due to slight variations in the functional residual air *from one breathing period to another.* This value was determined each day as the last measurement. It could have been determined for each breathing period by prolonging the oxygen breathing and collecting the remainder of the expired nitrogen in a separate gasometer. However, in such a procedure, no alveolar sample could have been obtained.

Small variations in tidal air from breath to breath introduce another source of error, which, however, is smaller than might be expected. Actual calculation shows that on experiments using more than 10 breaths of oxygen, a variation of 50 per cent about the mean tidal air leads to an absolute error of less than 2 per cent in the value of the predicted nitrogen concentration. When the number of breaths was less than 10, the tidal air was usually quite constant, provided sighs were suppressed.

Possible simplification for a practical test

In order to reduce unnecessary labor in any practical test it is important to know at what number of breaths (n), the deviation from the predicted value is maximum. With this information available, only 1 or 2 determinations would be necessary to evaluate intrapulmonary mixing.

An approximate mathematical relationship can be derived for this and then tested on some of the experiments. In its simplest form, the "prediction formula" is

$$x_n = Kr^n.$$

The actual mixing curve may be considered to follow a similar formula with a slightly larger dilution ratio (r′),

$$i.e., \quad x_n = K(r')n.$$

The difference between them can be expressed, $y = x' - x = K[(r')^n - r^n]$. Differentiating, setting $\frac{dy}{dn} = 0$ for a maximum and solving,

$$n = \frac{\log \log r - \log \log r'}{\log r' - \log r}.$$

Solving this for the various values of r commonly found, substituting a slightly larger value for r′ (actually r′ taken = r + 0.001), it was *found that the value of "n" best for the purpose* of showing divergence varies, as in the accompanying Table II.

TABLE II

Values for the best choice of number of breaths (n) calculated to show the greatest divergence from predicted curves

r	Optimum *n*
0.75	4
0.80	5
0.85	6
0.90	10
0.93	13
0.95	20
0.96	25
0.97	34
0.98	51
0.99	105

Let us apply this table to 2 of the abnormal subjects. In subject 8, r averaged about 0.85 and the maximum of the curve is obviously at 10 or less. In subject 10, r equals about 0.98; *the maximum is at 40 or higher.* Thus, as a rough approximation, the formula holds true. *Since the usual value for r is* 0.90 ± 0.05 for almost all subjects, it seems wise to pick the point of n = 10 as the most likely to show the effect of slow mixing, in any single test, which may be advised. If more points are possible, n = 20 and 40 could be used.

If constant underventilation of significant portions of the lungs occurs in normal individuals, one must imagine some mechanism whereby the blood circulation of these portions is simul-

taneously reduced. Otherwise, an oxygen unsaturation of the mixed arterial blood would result. It seems likely, however, that serious underventilation of normally perfused areas does exist in patients with severe pulmonary disease and is undoubtedly one of the causes of arterial anoxemia in such subjects. Thus, in a given patient with arterial anoxemia, tests such as we have presented will help to explain the cause of the disability. A positive test for poor mixing would point to such a mechanism as the cause of the anoxemia and would logically indicate the therapeutic use of bronchodilator drugs. A negative test would point to poor diffusion between alveoli and blood, to central depression of ventilation, or to some other cause of anoxemia. Appropriate tests might decide among these and other logical treatment might be indicated.

Possible mechanisms of poor mixing

At least two theories to explain incomplete intrapulmonary mixing in normal lungs have been proposed: (*1*) That of Sonne based on anatomical differences in elasticity, and (*2*) that of Engelhardt who suggests a physiological alternation of function of the various lung units. It is doubtful whether the proponents' experiments or the data here presented can adequately prove either theory. Possibly a more fruitful experiment would be to compare the mixing during voluntary hyperpnea with that during the increased ventilation of exercise. A difference between the two would favor some form of physiological adjustment over a purely anatomical cause.

While we can say very little about the exact mechanism of poor mixing, it is useful to inquire what the deviations from the curves in our data represent in terms of percentage failure of ventilation. It should be realized that the term "percentage failure of ventilation" is an arbitrary one. Actually, all degrees of relative under- and overventilation probably exist in the many lung units. Yet, for an arbitrary measure, we may calculate it as though at each breath a proportion of all units is completely shut off and the remaining units share equally in the new breath.

Considering the situation for a single breath of oxygen and using the same symbol and formulae (*A*) as in the introduction,

I. $(a - b)r' + b =$ actual lung nitrogen concentration.

In this case, r' is the value representing the actual dilution rate per breath and so may not equal the theoretical dilution rate $r = \frac{R}{R + T'}$. However, we may consider $r' = \frac{R}{R + T''}$. T'' in this case is a value obtained arbitrarily to fit the measurement. It has no real meaning but in case of imperfect mixing it will be smaller than T', the effective tidal air measured by Bohr's formula.

If α portion of the lung is ventilated, then

II. N_2 concentration of that portion $= \frac{\alpha R}{\alpha R + T'}(a - b) + b$ (using formula (*A*) of introduction).

III. N_2 concentration of unventilated portion $= a$ (by definition of "a").

IV. Total N_2 content of lungs $= \left[\frac{\alpha R}{\alpha R + T'}(a-b)+b\right]\alpha R + a(1-\alpha)R.$

V. Dividing by R, average lung nitrogen concentration $= \left[\frac{\alpha R}{\alpha R + T'}(a - b) + b\right]\alpha + a(1 - \alpha) = (a - b)\frac{R}{R + T''} + b$ (from equation I).

VI. Dividing by $(a - b)$,

$$\frac{\alpha R}{\alpha R + T'} + 1 - \alpha = \frac{R}{R + T''}.$$

VII. Solving for α, $\alpha = \frac{T'T''}{R(T' - T'') + T'T''}$.

It will be seen from the final formula that a small difference in T' and T'' will have much greater significance, the smaller the tidal air, and, conversely, the effect of imperfect mixing will be much more easily measured, the larger the tidal air.

Although this formula involves several additional assumptions when applied to experiments of more than one breath, these are probably preferable to the extreme complexities of any other mathematical treatment. Actually, the chief assumptions are that the portion ventilated is

constant at each breath and that the ventilated units occur by chance throughout the lung from both the previously ventilated and unventilated portions. Since these assumptions are more nearly those of the theory of alternation of function than the anatomical theory based on differences in elasticity, it follows that a constant α value would be a bit of evidence toward the theory of alternation of function, whereas a decreasing α value would favor the presence of constantly underventilated areas.

The following brief table gives the calculated α values in subject 10.

n	
20	4.1 per cent
40	3.4 per cent
80	3.0 per cent
120	2.9 per cent

The striking feature is the very low value for the ventilated portion (α) as calculated in this manner and the tendency for α to decrease with increase in the number of breaths in the experiment. This latter point fits with the known pathology of emphysema in which there are constantly underventilated lung regions.

Likewise, in subject 6 among the normal subjects, the following table shows similar trends, to a less striking degree. Possibly the decreasing values of α indicate some undiagnosed emphysema.

n	
1	54.7 per cent
2	36.1 per cent
3	50.8 per cent
5	48.0 per cent
7	37.6 per cent
10	30.0 per cent
15	27.2 per cent
20	20.9 per cent

The sensitivity of the methods in detecting incomplete mixing

It is striking that the ventilated lung portion (α), less than 60 per cent in subject 6, is calculated from a difference between T′ and T″ of only 50 cc. Unfortunately, the method as presented here cannot detect small or moderate degrees of unequal ventilation, especially when the tidal air is small. Let us take a hypothetical case (approximating subject 3) where the tidal air is 300 ml. and the functional residual air 2500 ml. Here a difference of only 10 cc. between T′ and T″ would lead to an α value of 50 per cent.

It is now obvious that, to measure accurately the degree of poor mixing, the tidal air must be large. In those cases in which it is naturally large, the effect may be evident from natural breathing. In other cases, it will be necessary to study the measurements on voluntarily deepened breathing. Such a study is being undertaken.

SUMMARY AND CONCLUSIONS

1. The effectiveness of the process of mixture of inhaled tidal air with the air already present in pulmonary spaces has been studied by means of the analysis of the respiratory gases during the breathing of pure oxygen, in normal subjects and in patients with pulmonary emphysema.

2. If intrapulmonary mixing of air is perfect, then the concentration of nitrogen in the lungs at the end of any given number of breaths of pure oxygen can be calculated when (*a*) the effective tidal air volume, (*b*) functional residual air volume, (*c*) initial "alveolar" (intrapulmonary) nitrogen concentration, and (*d*) rate of washing out the dissolved nitrogen in the body, are known. The formula describing this phenomenon has been derived.

3. Values for pulmonary concentration of nitrogen after breathing oxygen have been measured on 18 normal subjects and 5 patients with severe pulmonary emphysema.

4. For normal subjects, the divergence between predicted and measured values was small in the majority, but moderately great in a few. Difficulties in accurate measurement of the upper pulmonary dead space make further clarification difficult, but imperfect intrapulmonary mixture in some normal subjects is probable.

5. Four out of 5 emphysema subjects showed markedly higher nitrogen concentrations in the lung than predicted, indicating a marked degree of unequal lung mixture. It was possible to demonstrate, in actual clinical cases, the distinction between inadequate ventilation of pulmonary spaces due to greatly increased residual air, and that due to unequal mixture of tidal air through these spaces.

6. An estimation of the extent of underventilation of pulmonary spaces, in successive samples of alveolar air from normal and diseased subjects, provided some evidence against the existence of

any systematic alternation of function of discrete groups of alveoli.

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fication of protoporphyrin (11), for its conversion to mesoporphyrin (12), and for the quantitative determination of the erythrocyte protoporphyrin by means of the photoelectric colorimeter (13). The present study was undertaken primarily with the purpose of making simultaneous fluorescyte and reticulocyte counts, and determining the erythrocyte protoporphyrin, in a series of blood samples having different reticulocyte percentages and representing various clinical states. In spite of careful attention to the construction of a fluorescence microscope in accordance with Seggel's description (10), we have thus far been unsuccessful in our attempts to visualize fluorescytes satisfactorily. The study has necessarily been limited, therefore, to a comparison of reticulocyte percentage, at various levels and under different conditions, with the amounts of protoporphyrin in the erythrocytes, together with certain additional observations relating to the protoporphyrin alone.

EXPERIMENTAL

The erythrocyte protoporphyrin was determined by means of the method which we have recently described (13), which utilizes the Evelyn photoelectric colorimeter. For the most part, this method was applied only to the red blood cells. The most satisfactory procedure is as follows: A sample of whole blood is measured and centrifuged. The plasma is discarded. After washing once with physiological saline, the cells are subjected to the procedure previously described. The hematocrit percentage of the whole blood is determined separately in a Wintrobe tube. From this is calculated the volume of red blood cells used for the determination.

The reticulocyte preparations consisted of thin smears of blood superimposed upon and simultaneously mixed with thin films of brilliant cresyl blue on ordinary clean glass slides. The films of the dye were made simply by smearing the slide evenly with a 1 per cent alcoholic solution, and allowing it to dry. Immediately after superimposing the blood smear, the slide is placed in a moist chamber and allowed to stand for 10 minutes to permit supravital staining. After this, it is at once whipped dry. The circular aperture of the microscope ocular to be used is reduced to a square about one-sixth of the original size simply by inserting into the upper chamber of the ocular a round piece of paper with a square cut out of the middle. To further facilitate counting, this square is subdivided into four by two hairs laid at right angles across it, and held in place with glue. With an oil immersion lens, a total of 1,000 erythrocytes is counted and the percentage of reticulocytes noted. Differential reticulocyte counts, according to Heilmeyer's method (14), were also made. This method subdivides the reticulocytes into 4 groups, the relative maturity being indicated by the amount of reticulated substance noted. (For more exact orientation, reference may be made to the illustration in Heilmeyer's paper.)

RESULTS

1. The first part of the study consisted of observations on 5 rabbits in which acute phenylhydrazine anemia was produced by subcutaneous administration of 50 mgm. of phenylhydrazine hydrochloride, dissolved in 1.5 cc. of physiological saline. The blood for serial analyses was obtained by cardiac puncture. Potassium oxalate was used as anti-coagulant. The data obtained are shown in Figure 1. The values for the differential reticulocyte counts in 4 of the 5 rabbits are given in Table I. The data for the fifth will be omitted in order to conserve space. These data were essentially the same as in the 4 that are given. In experiments d and e in Figure 1, it is seen that the reticulocyte response is smaller in relation to the amount of protoporphyrin than was true in the other 3 animals. The possibility was considered that the reticulocyte response was not as great because of the fact that these 2 rabbits were considerably older than the remaining 3

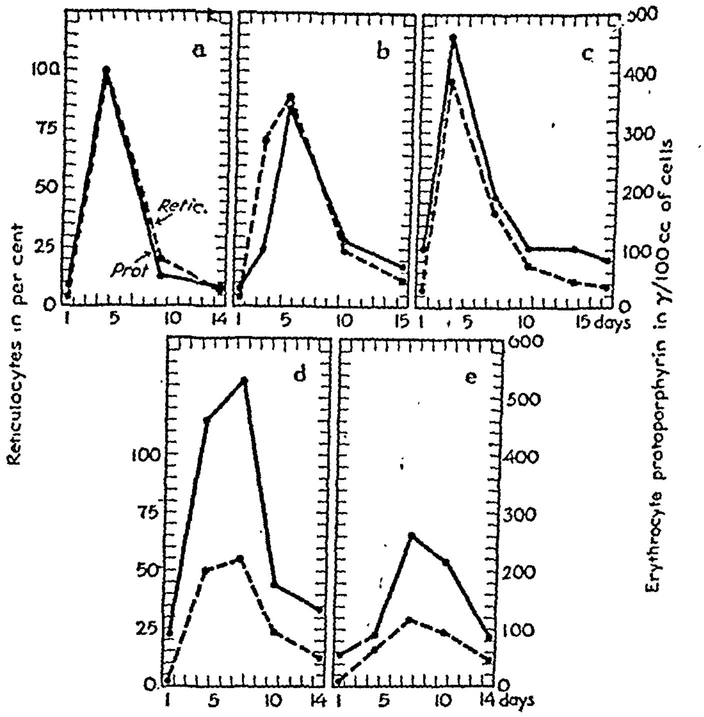

Fig. 1. Erythrocyte Protoporphyrin Concentration and Reticulocyte Percentage in 5 Rabbits (a to e) with Acute Phenylhydrazine Anemia

TABLE I

Differential reticulocyte counts, according to Heilmeyer, in rabbits with acute phenylhydrazine anemia

The determinations before administration are given in the column under 0 days. All of the values given are percentages.

	Rabbit	A						B				
		Days						Days				
		0	3	8	13			0	2	4	9	14
Increasing maturity of reticulocytes from I to IV	I	0.1	0.2	0.3	0.0			0.1	2.4	6.6	1.0	0.4
	II	0.5	16.6	3.5	0.1			0.5	5.4	13.2	5.3	1.0
	III	1.0	50.6	9.0	2.4			1.1	13.6	29.0	11.0	4.4
	IV	1.2	28.6	6.8	3.8			1.6	49.8	39.0	7.4	5.2
	Total	2.8	96.0	19.6	6.3			3.3	71.2	87.8	24.7	11.0
	Rabbit	C						D				
		Days						Days				
		0	2	6	9	13	16	0	3	6	9	13
Increasing maturity of reticulocytes from I to IV	I	0.2	1.2	0.6	0.6	0.1	0.1	0.0	0.6	0.2	0.2	0.0
	II	0.9	6.4	3.0	1.1	0.6	0.5	0.1	2.6	6.4	1.5	0.2
	III	2.0	56.0	14.8	7.2	3.9	2.5	0.6	7.8	17.2	10.2	4.9
	IV	3.5	33.2	22.8	9.4	5.5	6.5	1.2	38.0	30.0	11.7	7.5
	Total	6.6	96.8	41.2	18.3	10.1	9.6	1.9	49.0	53.8	23.6	12.6

TABLE II

Total and differential (Heilmeyer) reticulocyte counts, and erythrocyte protoporphyrin content, in a dog with acute phenylhydrazine anemia

Six hundred mgm. of phenylhydrazine hydrochloride were given in divided doses during the first 5 days. The values given for the reticulocytes are percentages in all instances.

	Group	Days				
		0	4	6	9	19
Increasing maturity of reticulocytes from I to IV	I	0.1	0.6	4.0	0.8	0.2
	II	0.2	2.5	10.0	1.8	0.9
	III	0.3	4.7	16.0	5.2	2.1
	IV	0.5	9.1	12.0	8.1	3.0
	Total	1.1	16.9	42.0	15.9	6.2
Protoporphyrin in γ per 100 ml. of whole blood		14.0	23.0	51.0	30.0	20.0

in the group. The difference could not be related to a higher percentage of younger reticulocytes (Table I). In general, the time correlation between reticulocyte and protoporphyrin increase and decrease, is seen to be very close. The same parallelism was noted in 1 experiment in a dog (Table II).

The combined protoporphyrin solutions, from all of the above experiments, were subjected to the chromatographic method of purification, as described in Paper I of this series. Precipitated calcium carbonate powder (Cenco) was used as adsorbent, and benzene-petroleum ether (1:1) as solvent. The chromatogram was developed with benzene alone. Crystalline protoporphyrin 9 dimethyl ester (M.P. 219° C.) was isolated (Figure 2). The spectroscopic properties were identical with those of pure protoporphyrin, as obtained from hemoglobin by the method previ-

FIG. 2. CRYSTALS OF PROTOPORPHYRIN 9 DIMETHYL ESTER (ISOMER TYPE III) AS ISOLATED FROM ERYTHROCYTES OF RABBITS WITH ACUTE PHENYLHYDRAZINE ANEMIA

ously described (11). This confirms the report of Grotepass (3) that the erythrocyte-protoporphyrin is the type 9 isomer. It may be noted that Grotepass' isolation was from normal human erythrocytes.

2. The second part of the study consisted in observing the amount of protoporphyrin in the erythrocytes, and the reticulocyte percentage, in 8 cases of pernicious anemia, before and at various periods after intramuscular injection of concentrated liver extract. The data obtained from the first 7 of these are shown in Figure 3, while those from the eighth case, which was studied recently, are given in Figure 4. It is seen that there is a lack of any close correlation, and, in several instances, even a marked divergence of the reticulocyte and protoporphyrin curves. The differences noted are obviously not related to variations in percentage of younger reticulocytes (Table III). In general, the peak in the proto-

TABLE III

Values for differential reticulocyte percentages, hemoglobin, red blood cells, and color index in pernicious anemia cases, before and after parenteral liver extract therapy

Color indices were calculated on the basis of 17 grams of hemoglobin per 100 cc. and 5,000,000 red blood cells per c. mm. Hemoglobin determinations were made with the Evelyn photoelectric colorimeter.

Case		A							B					C			
		Days							Days					Days			
		0	3	5	7	8	9	15	0	2	6	12	15	0	9	15	18
Increasing maturity of reticulocytes from I to IV	I	0.5	4.7	4.5		0.7		0.1		3.2	0.7	0.0	0.0				
	II	0.3	2.0	6.3		1.7		0.8		3.1	3.5	0.5	0.4				
	III	0.4	1.3	16.1		4.8		1.0		12.1	24.0	4.8	4.9				
	IV	0.4	0.1	5.5		8.8		1.9		1.4	32.1	9.0	3.8				
Total		1.6	8.1	32.4		16.0		3.8	1.1	19.8	60.3	14.3	6.1				
Hemoglobin in grams per 100 cc.		3.6	3.6		4.6		4.8	7.65	3.12			5.5	7.0	6.6	7.6	8.0	8.8
Red blood cells in millions per c. mm.		0.97	1.35		1.49		1.47	2.65	0.95			1.5	2.0	1.46	1.91	2.8	2.83
Color index		1.1	0.8		0.9		0.96	0.85	0.95			1.0	1.0	1.3	1.2	0.84	0.93

Case		D						E						
		Days						Days						
		0	3	7	12	14	15	0	1	4	6	7	12	15
Increasing maturity of reticulocytes from I to IV	I	0.3	0.4	0.1		0.0			0.2	0.1		0.0	0.0	
	II	2.1	2.1	0.4		0.0			0.2	0.3		0.1	0.0	
	III	6.1	3.3	1.5		0.9			2.0	7.8		3.8	1.0	
	IV	7.3	13.5	1.4		1.5			4.8	24.3		6.8	2.5	
Total		15.8	19.3	3.4		2.4			7.1	32.5		10.7	3.5	
Hemoglobin in grams per 100 cc.		3.98			10.0		11.0	4.2			6.0	7.8		11.0
Red blood cells in millions per c. mm.		0.96			2.4		2.45	1.1			2.5	3.2		3.5
Color index		1.2			1.2		1.3	1.1			0.7	0.72		0.93

Case		F						G						
		Days						Days						
		0	3	5	7	9	15	0	3	5	8	11	15	18
Increasing maturity of reticulocytes from I to IV	I	0.0	2.2	4.4	0.0	0.2	0.0			0.6	0.1	0.2	0.0	
	II	0.5	2.9	13.6	1.2	1.0	0.0			1.4	0.6	0.5	0.3	
	III	2.0	3.1	41.4	13.6	9.0	1.6			13.6	6.1	3.1	2.5	
	IV	3.0	5.4	18.8	42.2	21.8	6.0			26.6	16.2	8.6	4.5	
Total		5.5	13.6	78.2	57.0	32.0	7.6	5.2		42.2	23.0	12.4	7.3	
Hemoglobin in grams per 100 cc.		2.7					9.5	4.8			7.0	7.1		10.5
Red blood cells in millions per c. mm.		0.8					2.0	1.15			1.5	1.94		2.2
Color index		1.0					1.4	1.2			1.3	1.1		1.4

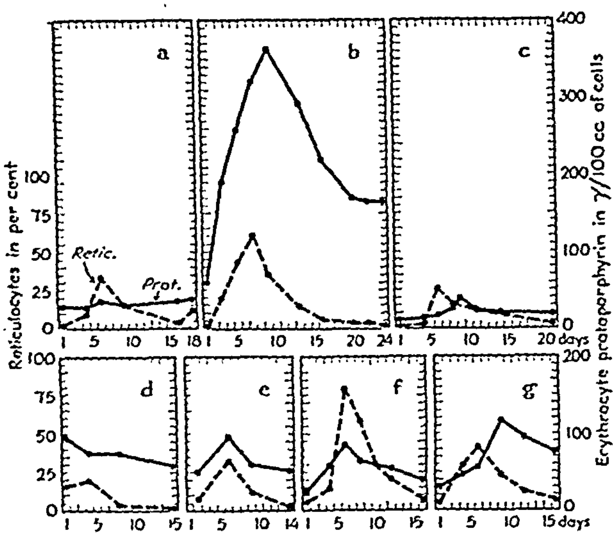

FIG. 3. ERYTHROCYTE PROTOPORPHYRIN CONCENTRATION AND RETICULOCYTE PERCENTAGE IN 7 CASES (a to g) OF PERNICIOUS ANEMIA, AS AFFECTED BY LIVER EXTRACT THERAPY

In each instance an amount of 30 to 40 U.S.P. units of anti-anemic substance was injected intramuscularly on the first day.

porphyrin curve is attained later than that of the reticulocytes. This is particularly well shown in Figure 4. The possibility must be considered that this delayed rise of the protoporphyrin is due to a developing iron deficiency. Our data on this point are somewhat fragmentary, but judging from the color indices given in Table III, and the hemoglobin concentrations shown in Figure 4, there is no evidence of any appreciable iron deficiency. Only in case e (Figure 3) was there any considerable decrease of the color index, and it may be noted that it later returned to the normal range without iron therapy, so that the significance with respect to iron deficiency is questionable.

It is noteworthy that the protoporphyrin values in the pernicious anemia cases prior to treatment were either low or normal, as compared with the data for supposedly normal individuals (Table IV). Furthermore, the increases following liver extract therapy were for the most part relatively small. The pernicious anemia data in Figure 3 and 4 may also be compared with those obtained in various diseases as shown in Table V. We do not consider that the values given in Table IV necessarily represent the normal in all instances.

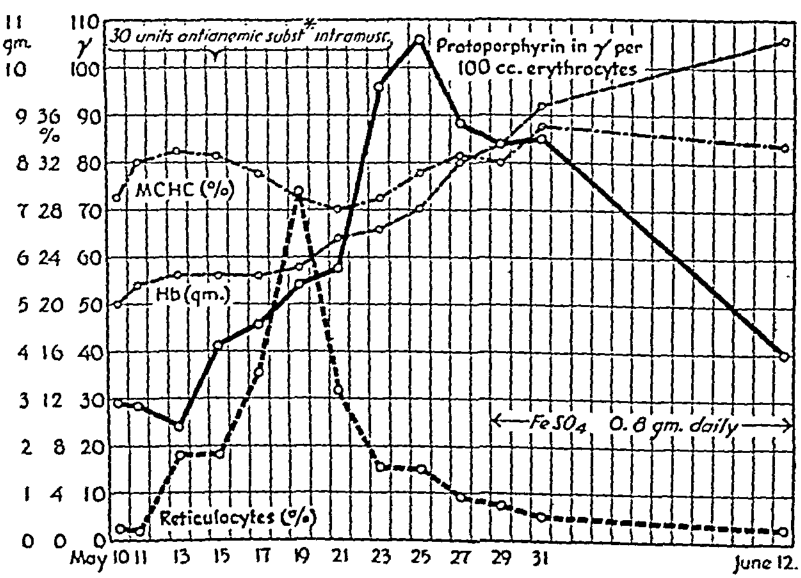

FIG. 4. ERYTHROCYTE PROTOPORPHYRIN CONCENTRATION, RETICULOCYTE PERCENTAGE, HEMOGLOBIN IN GRAMS PER 100 CC. OF BLOOD, AND HEMOGLOBIN CONCENTRATION OF THE ERYTHROCYTES, BEFORE AND AFTER LIVER EXTRACT THERAPY IN A CASE OF PERNICIOUS ANEMIA

C. S., M, 53.

* Liver concentrate, 10 units per cc., intramuscularly, on May 10th; this dose was repeated on May 27.

TABLE IV

Erythrocyte protoporphyrin in supposedly normal individuals (interns and nurses ranging in age from 20 to 25 years)

Number	Sex	Hematocrit percentage	Erythrocyte protoporphyrin
1	F	37.2	40.3
2	F	37.6	45.7
3	F	36.8	31.3
4	F	40.1	34.9
5	M	48.0	22.0
6	M	43.0	26.7
7	M	45.7	22.0
8	M	48.0	19.7
9	M	46.0	27.2
10	M	44.0	23.8
11	M	47.3	35.9
12	M	45.2	30.9

The 4 females included in this group were all student nurses, and it is noteworthy that a mild reduction of hematocrit percentage was present in 3 of these. It may be that the lowered hematocrit values are correlated with the increased amounts of erythrocyte protoporphyrin, these latter being somewhat greater than those observed in the males (healthy interns). Since mild anemia in otherwise normal young adult females is commonly related to increased menstrual blood loss, a further study of the erythrocyte protoporphyrin, with respect to the menstruation factor, is desirable. The data for the cases of post-hemorrhagic anemia, as seen in Table V, clearly reveal considerable increases.

3. The third part of the study was concerned with incubation experiments. Blood samples were drawn under sterile precautions from the animals and patients, as noted in Table VI, a and b. Potassium oxalate was used as anti-coagulant. The protoporphyrin content was determined at once on one-half of each sample, while the other half was incubated at 37° C. for 24 to 48 hours, after which, the amount of protoporphyrin was determined again. Since the volume of the erythrocytes increases upon incubation, it was necessary to determine the hematocrit percentage both before and afterwards and to make correction for the percentage increase in volume. In each instance, it was determined by culture that no growth of bacteria had occurred. This comparison before and after incubation was made primarily with the thought that if the protoporphyrin were fundamentally related to the *substantia reticulo-filamentosa,* a sharp diminution in amount

TABLE V

Erythrocyte protoporphyrin and reticulocyte percentage in various pathological states

Case	Sex	Age	Diagnosis and remarks	Protoporphyrin in γ per 100 cc. of erythrocytes	Reticulocyte percentage
1. A. B.	F	48	Hypochromic anemia; menorrhagia	153.0 178.0	2.0 4.1
2. S. D.	M	58	Hypochromic anemia; bronchial bleeding (after multiple transfusions)	277.0 95.0	5.4 3.3
3. A. H.	M	60	Hypochromic anemia; bleeding duodenal ulcer	221.1	0.9
4. H. B.	M	63	Hypochromic anemia; bleeding duodenal ulcer	103.4	1.2
5. M. J.	F	50	Hypochromic anemia; bleeding duodenal ulcer	50.0	0.7
6. I. S.	F	65	Advanced hepatic cirrhosis with moderate jaundice and anemia. Ascites. No bleeding. Color index 1.0	115.0	4.2
7. C. Q.	F	54	Cirrhosis with chronic jaundice and mild anemia. M. C. C. 35 per cent. Color index 1.0	34.5	
8. H. C.	F	41	Primary common duct cancer with complete biliary obstruction. No bleeding. Color index 1.0	69.0	5.8
9. L. L.	M	67	Leukemia with myelopthisic anemia, after many blood transfusions. Color index 1.0	43.0	0.0
10. G. S.	F	55	Pernicious anemia, untreated	10.0	2.2
11. S. T.	F	60	Pernicious anemia, untreated	24.0	3.4

TABLE V

Erythrocyte protoporphyrin in various pathological states, continued.

Case	Sex	Age	Diagnosis and remarks	Date	Protoporphyrin in γ per 100 cc. of erythrocytes	Reticulocyte percentage	Hemoglobin in grams per 100 cc.	Hematocrit percentage	M.C.H.C. percentage
12. A. H.	F	63	Macrocytic (acquired) hemolytic anemia	May 1, 1943	87	31	6.75	23	29
				May 5, 1943	78.2	30	6.00	23	26
				Blood transfusions					
				May 11, 1943	105.8	14.8	8.75	28	31
				May 13, 1943	99.9	14.8	9.25	29	32
				May 15, 1943	54.1	9.1	9.7	29.8	32
				May 17, 1943	56.3	7.8	11.7	32.0	36
				Splenectomy May 17, 1943					
				May 27, 1943	56.0	9.9	7.45	24.0	31
13. F. L.	F	44	Post-hemorrhagic, hypochromic anemia due to bleeding hemorrhoids. Iron therapy commenced on May 18, 0.8 grams $FeSO_4$ daily from that date on	May 21, 1943	613.0	6.2	5.75	23.0	25
				May 23, 1943	623.0	6.0	6.68	24.0	27.8
				May 25, 1943	600.0*	6.8	6.68	24.1	27.8
				May 27, 1943	545.4	6.0	6.5	25.0	26.0
				May 29, 1943	427.0	7.1	8.0	26.2	30.0
				June 1, 1943	407.0	7.5	8.05	29.0	28.0
				June 3, 1943	266.6	5.3	9.3	30.0	30.0
				June 7, 1943	348.0	3.2	9.11	31.0	29.0
				June 10, 1943	121.0	4.0	11.1	31.0	35.0
				June 12, 1943	80.0	3.2	10.5	37.5	32.0
				June 15, 1943	73.0	2.1	11.4	35.0	32.0
				June 21, 1943	40.0	1.4	11.2	36.2	31.0
14. P. B.	M	63	Familial hemolytic jaundice	May 25, 1943	37.0	5.9	11.7	32	33
15. I. H.	F	38	Familial hemolytic jaundice	June 15, 1943	42.0	5.3	2.7	8	34

* The porphyrin on this date was shown to be wholly chloroform soluble (protoporphyrin).

TABLE V

Erythrocyte protoporphyrin in various pathological states, continued

Case	Sex	Age	Diagnosis and remarks	Date	Protoporphyrin in γ per 100 cc. of erythrocytes	Reticulocyte percentage	Hemoglobin in grams per 100 cc.	Hematocrit percentage	M.C.H.C. percentage
16. M. F.	F	38	Uremia due to primary hypertension. Hemolytic anemia: Feces urobilinogen 720 mgm. per day. Exitus *	April 28, 1943	432.0	10.0	6.2	22	29.0
				April 30, 1943	424.0	9.0	7.4	23	32.0
17. J. O.	M	53	Refractory anemia following gold therapy for arthritis	May 25, 1943	107	0.7	9.4	27	35.0
				June 22, 1943	115	1.0	8.0	26	31.0
18. B. H.	F	35	Subacute disseminated lupus erythematosus	June 9, 1943	29.0	0.3	10.8	35	31
19. M. R.	M	25	Chronic tertian malaria	June 22, 1943	69.0	6.1	13.5	38	35
20. F. B.	F	59	Chronic myeloid leukemia	June 22, 1943	56.4	0.0	6.5	17	38
21. A. B.	M	24	Intermittent acute porphyria during acute attack	June 8, 1943	22.0	0.2	13.5	40	34

* In this case, the bone marrow of the shaft of the femur was red and hyperplastic. Approximately 2 grams of this red marrow were first ground and mashed with physiological saline in a mortar, then extracted for protoporphyrin as in the method for erythrocytes (13). No fluorescence was demonstrable in the final HCl. The same was true of a similar amount of fresh spleen, similarly treated. From this, it would appear that the fixed cells of the bone marrow and spleen do not contain porphyrin. This would apply particularly to reticulo-endothelial cells and probably to early erythroblasts, but not to normoblasts.

might be expected during incubation, since it is well-established that the majority of the reticulocytes mature, or at least lose their ability to stain supravitally, after 24 to 72 hours (15). Surprisingly, however, it was found that the protoporphyrin of the erythrocytes regularly increased upon incubation. The data are given in Tables VI, a and b.

4. Further observations were made of the behaviour of dilute mixtures of brilliant cresyl blue and protoporphyrin. Watson and Clarke (4) noted that a precipitate was readily obtained from dilute solutions of the two substances, and that the microscopic appearance of this precipitate bore striking resemblance to the *"substantia reticulo-filamentosa"* in blood films, stained supravitally with brilliant cresyl blue. Present observations indicate that the mutual precipitation of the two substances may not be due to formation of an actual compound, as at first thought, but rather to an effect of the porphyrin upon the relative solubility of the dye in buffered solutions having a pH approximating that of the blood. Thus, the addition of 1 cc. of 0.2 per cent brilliant cresyl blue in physiological saline, to 5 cc. of a phosphate buffer solution of pH 7.3, results in the slow appearance of an almost imperceptible precipitate of the dye. In a solution, similarly prepared, but to which an amount of protoporphyrin barely sufficient to color is first dissolved in the Na_2HPO_4 which is to be employed in preparing 5 cc. (pH 7.3), the precipitate forms more rapidly and is more voluminous.

TABLE VIa

Increase of erythrocyte protoporphyrin during incubation of whole blood under sterile conditions

Subject	Time in hours	Reticulocyte percentage	Protoporphyrin in γ per 100 cc. of erythrocytes; *whole blood
Dog Acute phenylhydrazine anemia	0 24 48	16.9 7.0 2.8	23* 38* 46*
Rabbit Acute phenylhydrazine anemia	0 24		108 232
Rabbit Lead poisoning	0 24		116.5* 276.0*
M. S. F 68 Polycythemia vera	0 24 48		18.0 25.0 30.0
A. B. M 63 Polycythemia vera	0 24		55.0 71.5
A. B. F 48 Menorrhagia; hypochromic anemia	0 24		51.5* 61.0*
D. H. F 39 Macrocytic hemolytic anemia	0 24		58.0 64.0
C. F 28 Chronic ulcerative colitis; hypochromic anemia	0 24 48	7.6 5.3 1.8	64.0 96.0 106.0
E. K. F 62 Bleeding peptic ulcer; hypochromic anemia	0 24 48		118.0 129.0 140.0

DISCUSSION

The results obtained in acute phenylhydrazine anemia in rabbits (Figure 1) are quite in accord with the concept that the erythrocyte protoporphyrin resides in the reticulocytes. This is not supported, however, by the data obtained in the cases of pernicious anemia, nor in the incubation experiments (Figures 3 and 4, and Table VI, respectively). In both of these groups, it is apparent that the erythrocyte protoporphyrin may increase as the reticulocyte percentage diminishes. It is also evident from the data in Table V that there is a lack of correlation between reticulocyte percentage and erythrocyte protoporphyrin concentration in various conditions. These results make it clear that the problem of the erythrocyte protoporphyrin is much more complex than was indicated in the earlier study of Watson and Clarke (4). The conclusion reached at that time, *i.e.*, that the erythrocyte protoporphyrin "resided chiefly, if not solely, in the reticulocytes," was based upon a correlation of two observations: (*1*) The presence of the protoporphyrin in the reticulocyte-rich, upper strata of centrifuged blood, and (*2*) the presence of large amounts of protoporphyrin in samples of blood containing only reticulocytes. This conclusion appeared to be well grounded, and there is, in fact, no reason to doubt that under many circumstances, at least, the re-

TABLE VIb

Increase of erythrocyte protoporphyrin during incubation of whole blood under sterile conditions

Subject	Time	Reticulocytes	Blood incubated	Hematocrit	Erythrocytes calc. from hematocrit	Protoporphyrin	
						Erythrocytes	Whole blood
	hours	*per cent*	*cc.*	*per cent*	*cc.*	*γ per cent*	
E. V. M 47 Polycythemia	0	0.4	13.0	63.8	8.29	21.7	13.8
	24	0	13.0	74.8	9.72	38.0	27.5
	48	0	12.0	89.0	10.68	33.7	30.3
M. F. F 38 Hypertension; chronic uremia. Hemolytic anemia	0	9.0	15.0	22.0	3.3	424.0	93.2
	24	6.0	8.5	26.2	2.22	406.4	106.4
	48	0.0	7.5	32.0	2.4	416.6	132.1
A. H. F 63 Macrocytic hemolytic anemia	0	30.0	10.7	24.0	2.56	78.2	18.7
	24	28.0	10.8	30.0	3.24	74.0	22.0
	48	26.0	11.1	33.0	3.66	76.5	25.0
F. H. F 60 Pernicious anemia, untreated	0	0.9	10.2	23.4	2.38	23.1	5.4
	24	0.7	10.3	33.2	3.41	23.4	7.74
	48	0.2	11.8	35.0	4.13	24.2	9.9
C. S. M 53 Pernicious anemia, after treatment	0	12.6	10.2		2.42	57.8	14.0
	24	8.0	10.0		2.8	57.2	16.0
	48		9.4		2.9	65.5	19.0
W. E. M 61 Periarteritis nodosa	0	0.4	10.0		2.9	155.2	45.0
	24	0.4					
	48		10.0		4.6	184.0	80.0

ticulocytes are relatively rich in protoporphyrin. The available evidence now indicates, however, that there are at least two factors, in addition to erythropoietic activity, to explain the presence of protoporphyrin in the erythrocytes. The additional factors are believed to be: (*1*) Iron deficiency, and (*2*) formation of protoporphyrin from hemoglobin in intact erythrocytes.

The recent studies of Stasney and his co-workers (*16*) indicate that normoblasts contain protoporphyrin in considerable amount. This is in accord with the finding of protoporphyrin in the reticulocytes in experimental, acute phenylhydrazine anemia. It is also of interest to consider the present findings in pernicious anemia in relation to Stasney's observations on the porphyrin content of the bone marrow in this disease. Stasney was unable to find protoporphyrin in the megaloblastic marrow of pernicious anemia patients in relapse, but observed it in increasing amount as the marrow became more and more normoblastic, shortly after liver extract therapy. It should be pointed out that previous studies of the bone marrow porphyrin in pernicious anemia are not in accord. Thus, Borst and Königsdorffer (*17*) regularly noted protoporphyrin in the megaloblasts of pernicious anemia marrow, while Seggel and his co-workers were unable to find it, either before or after treatment. Nor could they observe it in any other condition (including hemolytic jaundice), with the one exception, however, of lead poisoning. The reports of both of these groups of investigators were based upon studies with the fluorescence microscope, while Stasney employed extraction of considerable amounts of marrow, and, after suitable fractionation, examined the final concentrated solution with ultraviolet light.

In the present study, the protoporphyrin values obtained for the circulating red cells in pernicious anemia, before liver therapy, were relatively low (Figures 3 and 4; Tables V and VIb). Increases are noted following treatment, but in most in-

stances the highest level was reached only at some time after the reticulocyte peak. The reason for this is not clear. The possibility exists that most or at least many of the reticulocytes at the peak were derived from megaloblasts, and hence, if Stasney's observations be correct, contained little or no protoporphyrin; later, as more and more of the young circulating cells are derived from normoblasts, the protoporphyrin content would be expected to increase. Obviously, much more study of this problem is needed, together with simultaneous quantitative data on the bone marrow porphyrins.

Seggel also observed little or no protoporphyrin in the circulating red cells during relapse, a finding which was correlated with a negligible number of fluorescytes (10). Seggel regarded the increased fluorescyte percentage occurring after the reticulocyte peak as being due to iron deficiency. In the present study, however, evidence of iron deficiency during this period is not convincing. If one falls back upon the rapid regeneration (incomplete synthesis) theory of Seggel's, it would be logical to expect the two curves to be parallel. This was true in but 2 of the 6 cases shown (cases e and f in Figure 3). It is quite possible, however, that this is the explanation for the parallel curves noted in Figure 1, where hemolysis and rapid regeneration were induced by phenylhydrazine.

Formation of protoporphyrin from hemoglobin in intact erythrocytes would appear to be the only explanation for the results of the present incubation experiments (Table VIa, b). That such a factor obtains *in vivo* has not been shown, but there is much reason to suppose that temporary sequestration of erythrocytes as, for example, in the splenic pulp, is comparable in many respects to sterile incubation *in vitro* (18 to 21).

The protoporphyrin value noted for the cases of leukemia with myelopthisic anemia (Cases 9 and 20 in Table V) in which no reticulocytes could be found, is likewise best explained on the basis of formation from hemoglobin. In neither of these cases was there any evidence of iron deficiency. The increased values observed in the hemolytic anemias may also be at least partly explained on this basis, while a part of the increase may be due, again, to incomplete synthesis on the basis of rapid regeneration. The considerable day to day fluctuation of amount, as noted in Case 12 (Table V), could be attributed to either mode of formation of the protoporphyrin.

Assuming that protoporphyrin may be formed from hemoglobin in intact erythrocytes is not to say that it is an intermediate stage in the formation of bilirubin, against which some evidence, at least for the dog, has been described previously (22). Bilirubin has been shown to increase upon sterile incubation of erythrocytes (23), but the available evidence (23 to 26) indicates the presence of a biliverdin iron-globin, rather than protoporphyrin, as the intermediate compound.

The third factor deserving consideration is that of iron deficiency. It is logical to assume that unused protoporphyrin might be present in red blood cells which are hypochromic because of iron deficiency. Seggel and his co-workers have provided considerable evidence for this view (10), and the present data (Table V) are in agreement. The effect of iron therapy is particularly well shown in Case 13 (Table V).

The clinical data given in Table V make it evident that determination of the erythrocyte protoporphyrin is likely to be of considerable interest in the study of anemias generally, both as to classification and underlying pathologic physiology. It is not inconceivable that the method will have value in the detection of incipient damage due to heavy metals and other toxic agents. We have not yet determined the concentration in human cases of lead poisoning, but the data of others (10, 27), together with our own observations in rabbits, are indicative of the marked increases which occur. The considerable increase noted in the case of refractory anemia following gold therapy (Table V) suggests that the method may have value in this direction also.

Seggel (10) regards the increased protoporphyrin content of the erythrocytes in lead poisoning as due to interference in hemoglobin synthesis. Kench and his co-workers (27) are not in accord with this view, since their data indicated that the increased porphyrin formation (both proto and copro III) is much too small to correspond with the total reduction in amount of hemoglobin. This objection may or may not prove valid, since it is difficult to exclude other factors relating to a decreased amount of hemoglobin.

One of the most interesting of the present cases

is that of the hemolytic, regenerative anemia associated with uremia (Case 16, Table V). The cause of the marked increase of erythrocyte protoporphyrin, but a few days before death, is not clear. One is tempted to assign it to a toxic hemolysis with associated disturbance of hemoglobin synthesis.

The present results do not appear to support the concept of an *in vivo* precipitation of brilliant cresyl blue and protoporphyrin as explaining the supravital staining phenomenon. This possibility was suggested by Watson and Clarke (4), but it was not proven, although others have subsequently stated, incorrectly, that Watson and Clarke's study proved identity of the basophilic substance of the reticulocytes with protoporphyrin (27). The data for the phenylhydrazine anemia rabbits, it is true, are quite compatible, and even suggestive, but the remaining studies, and especially the incubation experiments, are not in accord. The idea should probably not be entirely discarded, however, since it is conceivable that various factors might prevent an intracorpuscular protoporphyrin derived in one way from precipitating with the dye, while that formed in another way might readily do so. Protoporphyrin derived from hemoglobin, as in the incubation experiments, might not be effective, while that representing formative material not yet used in hemoglobin synthesis, as in the phenylhydrazine anemic rabbits, might easily serve to precipitate the dye during the supravital staining. In particular, the state of the erythrocyte protoporphyrin with respect to iron and protein under any given circumstances would merit further consideration in this respect.

SUMMARY AND CONCLUSIONS

1. In rabbits suffering from acute phenylhydrazine anemia, the curves of reticulocyte percentage and erythrocyte protoporphyrin content follow each other closely at various periods. The crystalline protoporphyrin, isolated from the erythrocytes in these experiments, was found to be isomer type 9, corresponding to aetioporphyrin III.

2. In pernicious anemia patients, studied before and at various intervals after liver extract therapy, the erythrocyte protoporphyrin curves usually reached their peaks only at some time after the maximal reticulocyte percentages. This delayed rise is not correlated with the relative age of the reticulocytes, as determined by the amount of *substantia reticulo-filamentosa.* The possibility is considered that the first mass of reticulocytes appearing after liver therapy may be derived from megaloblasts and may not contain as much porphyrin as those derived from the normoblasts and entering the circulation a short time later.

3. Correlation was not observed between the reticulocyte percentage and erythrocyte protoporphyrin content in a series of observations in various diseases. Increased amounts of erythrocyte protoporphyrin were regularly observed in post-hemorrhagic, iron deficiency anemias. The amounts observed in hemolytic anemias, and in certain toxic states, were likewise considerably elevated. By comparison, the values observed in untreated cases of pernicious anemia were relatively low, and were within the normal range.

4. Sterile incubation of various blood samples, for from 24 to 48 hours, revealed a regular increase of the erythrocyte protoporphyrin. This increase occurred in spite of a decreasing reticulocyte percentage. The possibility is considered that erythrocyte protoporphyrin may be formed *in vivo* from hemoglobin, under certain circumstances.

5. At least three factors are thus held likely for the presence of the erythrocyte protoporphyrin: (*1*) Normoblastic activity in the bone marrow; (*2*) iron deficiency or interference in utilization of iron in hemoglobin synthesis; and (*3*) hemoglobin degradation in intact red blood cells.

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VARIATIONS IN CUTANEOUS AND VISCERAL PAIN SENSITIVITY IN NORMAL SUBJECTS [1]

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A striking variation in the intensity of pain, experienced in diseases with apparently similar lesions, is a common observation. This fact may indicate a variation in pain sensitivity in different individuals, or a variation in pain sensitivity in a given person because of different environmental or physiological conditions. The amount of pain arising from lesions cannot be quantitatively measured. One must, therefore, employ the alternative of introducing a measured stimulus and, by the artificial production of pain, study individual responses under varying conditions. By such studies, one may hope to obtain an explanation of clinical variations in pain.

Numerous studies on pain sensitivity have been made (1 to 6). Prior to 1940, attempts to quantitate cutaneous pain sensitivity were limited chiefly by the technical difficulties of creating a stimulus which could be accurately measured and which produced a readily appreciated end-point of pain. The cutaneous heat-radiation apparatus, developed by Hardy, Wolff, and Goodell (6), overcame both of these difficulties. Visceral pain also has been studied experimentally by many investigators (7 to 9), but, so far as is known, no satisfactory quantitative measurements of visceral pain sensitivity have been made.

The present study constitutes an attempt to measure pain sensitivity in 200 normal subjects. They were considered to be normal in that they showed no evidence of physical or mental disease, either by examination or by history. Their ages ranged from 10 to 85 years. A majority of the group were of Northern European stock; the remainder included 25 Southern Negroes, 15 Ukrainians, and 30 of Jewish and other Mediterranean races. Various economic, occupational, and social groups were represented, as well as varying body types and emotional patterns. All subjects were tested for pain sensitivity by a modification of the heat-radiation apparatus of Hardy, Wolff, and Goodell. In addition, 29 of the group were tested for visceral sensitivity by balloon distention of the lower esophagus, and, in a smaller group, attempts were made to modify the initial pain sensitivity by the conditions of fatigue, nervous tension, acidosis, alkalosis, fasting, and by the administration of epinephrine and acetyl-beta-methyl-choline.

To determine an individual's pain sensitivity, we measured two manifestations of the pain experience: (*1*) the pain-perception threshold, which is a subjective end-point; and (*2*) the pain-reaction threshold, or the first objective evidence of withdrawal from the pain stimulus. In the study of cutaneous pain, both factors were measured. For visceral pain, only the pain-perception proved measurable, because no uniform pain sensation, or uniform pain-reaction end-point, could be elicited by balloon distention of the esophagus. The only readily recognizable end-point for visceral sensitivity was a sense of beginning substernal fullness, and while this end-point could not be considered strictly a pain sensation, for the purposes of this study, the point at which it was first noted was measured and called the visceral sensory threshold. The terms "visceral pain" and "visceral sensitivity" are used interchangeably.

CUTANEOUS PAIN SENSITIVITY

Method. As previously reported by Wolff and his associates (6), the stimuli for cutaneous pain consisted of varying intensities of light, which were focused on the middle of the subject's forehead by a heat-radiation apparatus. The source of the stimulus was a 1,000-Watt tungsten filament lamp, focused by two 4-inch planoconvex lenses through an aperture 2.5 $cm.^2$ in area. Each exposure was kept constant at 3 seconds by a shutter operated by a telechron motor. The intensity was varied

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in a uniform manner by a wire rheostat. The amount of heat used was measured directly by a radiometer and potentiometer and expressed in absolute end-point values of gram calories per second per square centimeter of skin surface. In order to prevent the reflection of wave lengths from the forehead, to minimize the penetration of these wave lengths into the skin, and to convert radiant into molecular energy, in which form the heat is conducted through the epidermis to the pain endings (10), the skin was blackened with India ink before placing the subject's forehead against the aperture. The cutaneous pain-perception threshold was held to be the smallest amount of heat stimulus from this apparatus sufficient to cause a sharp, jabbing sensation. The pain-reaction threshold was determined as the smallest stimulus necessary to cause the subject to wince, that is, a beginning contraction of the eye muscles at the outer canthus.

The conditions under which these two end-points were measured were standardized in the following manner. All tests were made by the same observer. Each subject was tested on at least three different occasions, at the same time of day, and in the same relation to meals. Fatigue and nervous tension, insofar as possible, were eliminated; no drugs or stimulants, other than tea or coffee, were taken. Each test consisted of from 10 to 14 exposures of light with a 2-minute interval between each exposure, making the total time for each test between 25 and 40 minutes. In order to avoid the error of suggestion, the description of the subject's varying sensations was elicited by 5 neutral questions, asked after each exposure:

1. "What did you feel?"
2. "How would you describe what you felt?"
3. "Was this one as intense, less intense, or more intense than the previous one?"
4. "Was the sensation you felt like any which you have felt anywhere on your body before?"
5. A card with 7 numbered circles, varying in size from a half-dollar to a pencil-point, was held before the subject and he was asked which circle corresponded to the size of the spot on his forehead where the stimulus seemed most intense. His answer gave an objective picture of the pain-perception end-point and usually indicated a circle the size of a pencil-point, although the skin area exposed remained constant at the size of the aperture.

At the end of each test, observations were noted as to any detectable modifying factors such as fatigue, nervous tension, or apprehension. The subject was finally asked these questions: "Were you nervous or restless during the test?", "Are you tired?", "How much sleep did you have last night?", "When was your last meal?", "Have you had any stimulants?" If the person tested was a female, the relation to her menstrual period was noted.

The second test was run in the same manner as the first except for 2 points. The subject was asked to keep his head at the aperture until the end of each exposure, which had not been required before, to see if this change in procedure would alter the stimulus level that had caused him to wince. The second point of difference was that during the latter half of the test he was told how the various sensations were commonly described, and in this way the effect of suggestion on his pain-perception threshold was checked.

When the tests were completed, the word "pain" was mentioned for the first time. The subject was asked to define pain and to state whether the initial sharp jab sensation, taken as the pain-perception end-point, seemed to him actually painful. His personal appraisal of pain sensitivity, gauged by experience (dental, labor, or menstrual pain), as well as by the frequency of his use of analgesics, was compared with the experimental observations.

Results. The results of the cutaneous pain measurements of 200 normal subjects showed a variation in pain-perception ranging from — 40 per cent to + 50 per cent of a mean average value of 0.305 gram cal. per second per sq. cm.[8] (Figure 1 and Table I). For a given individual, tested under standard conditions, the percentage variation during the tests was from ± 2 per cent to ± 6 per cent. The individual percentage variation was small compared with that observed for the entire group. The spread between the point at which pain was perceived and that at which wincing occurred varied from 0 (for the most reactive subject) to 50 per cent (for the least reactive subject). The average spread between pain-perception and pain-reaction for the group was 20 per cent (Figure 2). The individual percentage variation in pain-reaction ranged from 0 to 8 per cent.

Age and race were the only 2 factors which appeared to have a conclusive bearing on the variations of cutaneous pain-perception. Both pain-perception and pain-reaction decreased with age, although there were individual exceptions (Figure 3). The youngest age group, 10 to 22 years of age, had an average pain-perception threshold of 0.289 gram cal. per second per $cm.^2$. In the age group from 23 to 44 years, the mean value was 0.324 gram cal. per second per $cm.^2$ of skin surface; from 45 to 85 years, the main value was 0.347 gram cal. per second per $cm.^2$. The changes in pain-reaction according to age were parallel to those in pain-perception. The spread between pain-perception and reaction would have been

[8] For standard error determinations for this and subsequent comparisons, see Table I.

TABLE I.

Mean values and statistical constants

		Age groups	Number of subjects		Cutaneous pain thresholds: Perception	Cutaneous pain thresholds: Reaction
		years			*gram cal. per second per cm.^{2}*	
Normal controls (All races studied)		10 to 85	200	Mean	0.305	
				Range	0.175 to 0.462	
				S. D.*	0.045	
				S. E.	0.003	
Age comparison (Northern Europeans)		10 to 22	20	Mean	0.289	0.375
				Range	0.235 to 0.335	0.305 to 0.430
				S. D.	0.025	0.034
				S. E.	0.006	0.008
		23 to 44	20	Mean	0.324	0.406
				Range	0.283 to 0.400	0.325 to 0.457
				S. D.	0.032	0.034
				S. E.	0.007	0.008
		45 to 85	20	Mean	0.347	0.410
				Range	0.290 to 0.450	0.330 to 0.450
				S. D.	0.043	0.039
				S. E.	0.010	0.009
Racial comparison	Negroes	18 to 44	18	Mean	0.268	0.301
				Range	0.228 to 0.325	0.252 to 0.335
				S. D.	0.021	0.029
				S. E.	0.005	0.006
	Northern Europeans	18 to 44	18	Mean	0.318	0.384
				Range	0.264 to 0.410	0.280 to 0.480
				S. D.	0.036	0.049
				S. E.	0.008	0.012

Racial differences in the spread between pain perception and pair reaction

	Mean	Range	S.D.	S.E.
	gram cal. per second per cm.^{2}			
Negro	0.033	0.0 to 0.084	0.024	0.006
Northern European	0.066	0.016 to 0.180	0.032	0.008

Values for visceral sensory threshold

Number of Subjects	Mean	Range	S.D.	S.E.
	cm. of water pressure			
29	37	15 to 89	16.3	3.0

* S. D. = Standard Deviation. S. E. = Standard Error.

greater in the oldest age group had not the possibility of blistering the forehead prevented the giving of an adequate stimulus to establish the true reaction end-point. As indicated in Figure 3, these subjects were of the same sex and racial group.

For comparison of race variations, a series of 18 Negroes and a corresponding number of Northern Europeans, of the same age and sex groups, was studied. The Negro perceived pain at a lower level than the Northern European (Figure 4), but he reacted to pain at, or very

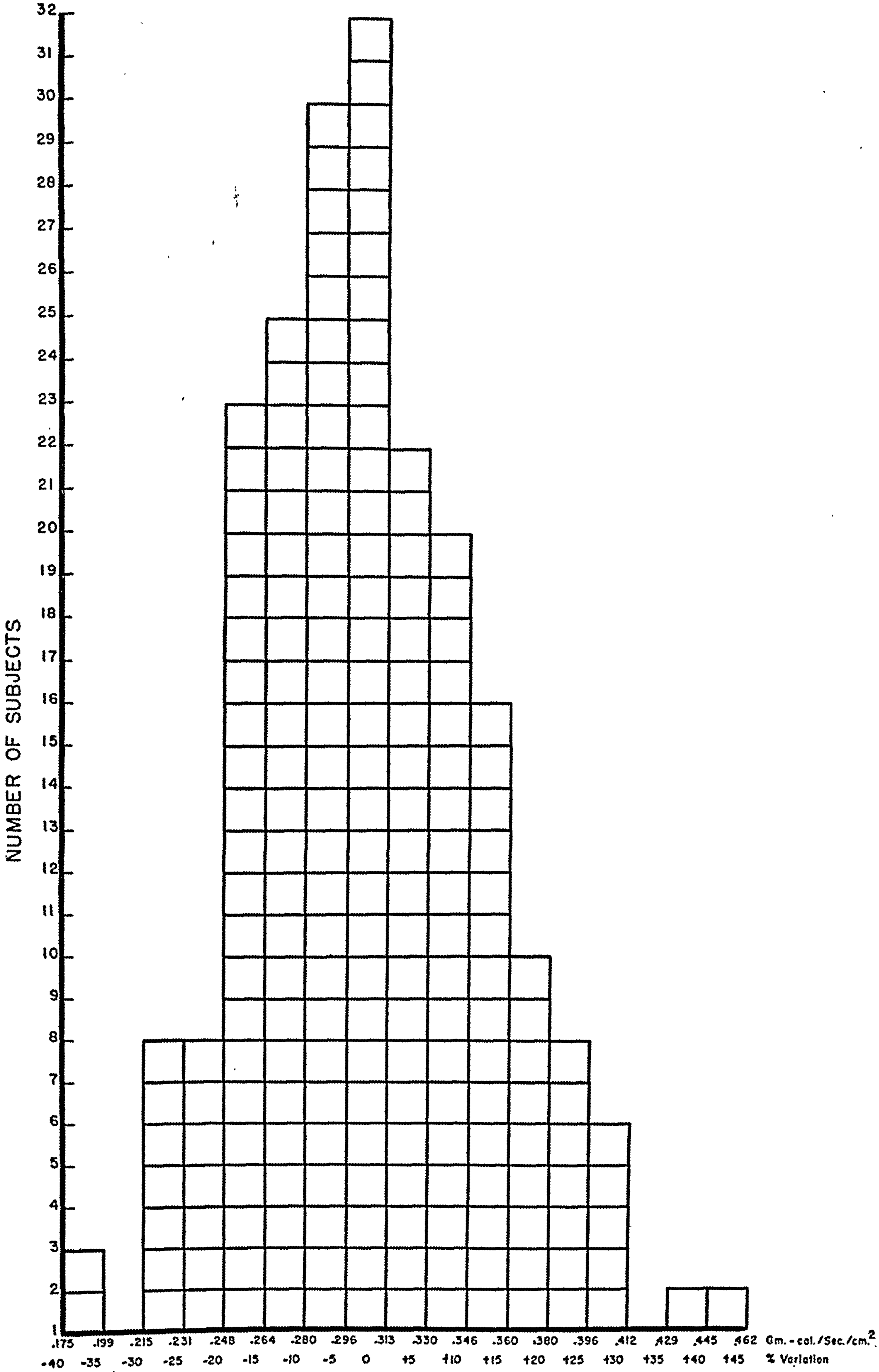

FIG. 1. CUTANEOUS PAIN-PERCEPTION THRESHOLD VARIATIONS IN 200 NORMAL INDIVIDUALS

Age range, 10 through 85 years. No racial segregation. Each square represents the average result of at least 3 separate determinations, carried out under standard conditions. Abscissa readings represent heat stimulation expressed in terms of gram calories per second per square centimeter skin surface. Percentage differences are expressed as plus or minus variations from the mean for the entire series.

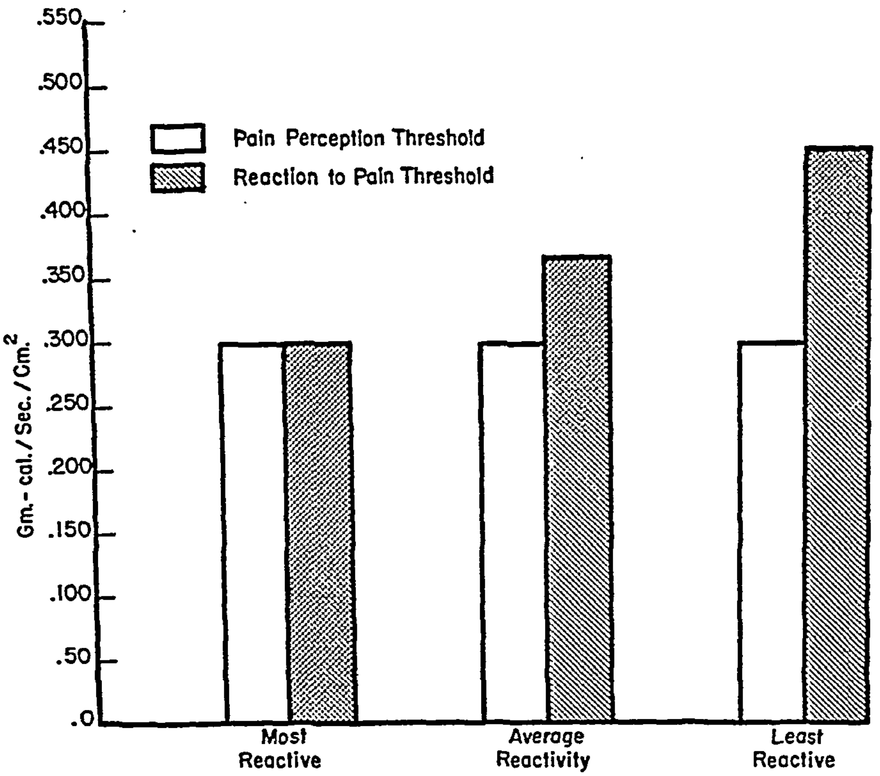

FIG. 2. RELATION BETWEEN CUTANEOUS PAIN-PERCEPTION AND PAIN-REACTION THRESHOLDS

This chart illustrates the minimal and maximal variations in pain-reaction, as contrasted with average normal difference noted in normal subjects. Note that in the most reactive subject, signs of wincing or withdrawal, which have been taken as the pain-reaction threshold, may occur at the same point of stimulation as that producing pain-perception. In the least reactive subject, there is a wide spread between the amount of thermal stimulation required to produce wincing and that producing pain-perception.

near, his pain-perception level, whereas the Northern European's spread between the perception and reaction levels was a very appreciable one. The Negro had a average pain-perception threshold of 0.268 gram cal. per second per cm.^{2}, as compared with an average of 0.318 gram cal. per second per cm.^{2} for the Northern European. The average spread between pain-perception and pain-reaction for the Negro was 0.033 gram cal. per second per cm.^{2}, as compared with 0.066 gram cal. per second per cm.^{2}, for the Northern European.

The group of Mediterranean races tested had both pain-perception and reaction values which corresponded more nearly to those of the Negro, with one difference,—the Negro revealed little or no overt response at the point of wincing or withdrawal, whereas the Mediterranean subject was apt to protest at being subjected to so intense a stimulus. On the presumption that the increased pigmentation of the Negro's skin might account for the differences in the pain measurements obtained, 3 other colored subjects with vitiligo were tested, comparing non-pigmented areas and corresponding pigmented areas in each subject. Since pain-perception and pain-reaction values for both areas were the same, it was concluded that the increased pigmentation of skin in the Negro could not account for the racial differences observed.

The difference between our findings and those reported by Wolff and his associates (11) is of interest. According to Wolff, the cutaneous pain-perception variation in a group of 150 normal

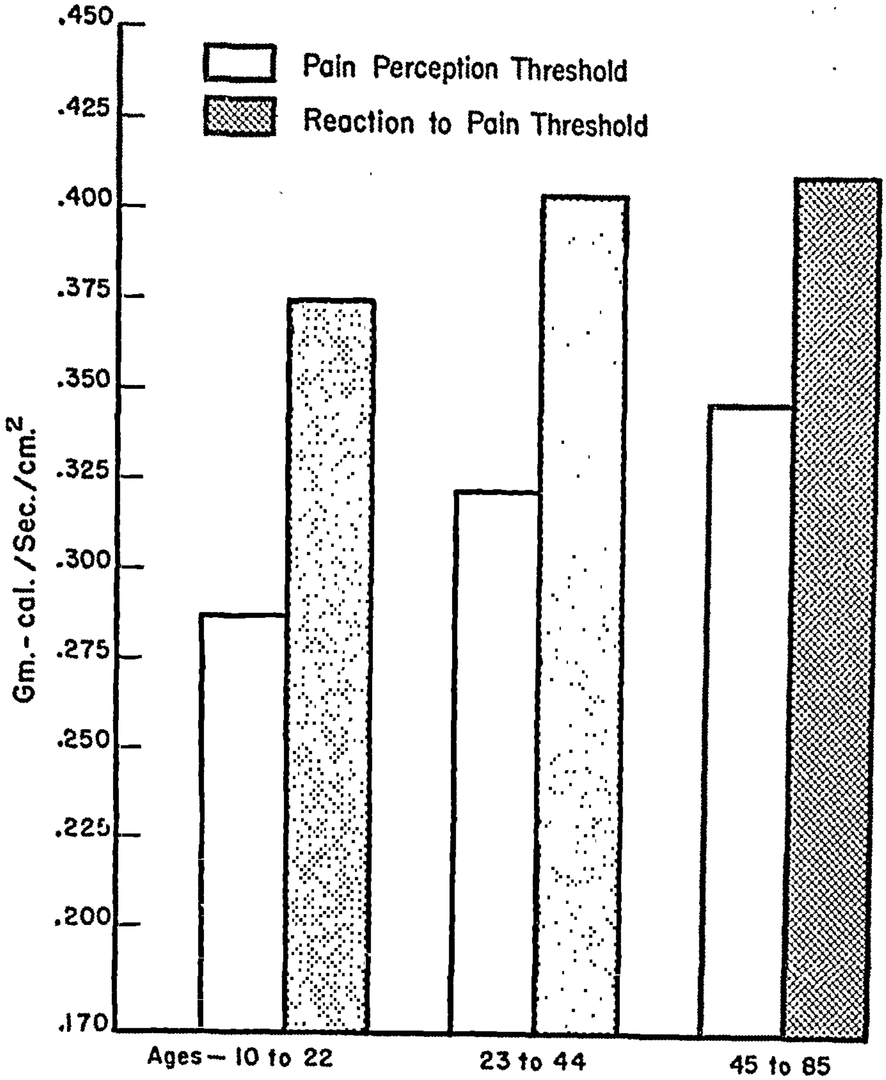

FIG. 3. RELATION OF CUTANEOUS PAIN-PERCEPTION THRESHOLD AND CUTANEOUS PAIN-REACTION THRESHOLD TO AGE

Subjects of same sex and racial group. The decrease in pain sensitivity with age is shown in the steady rise in mean average values for cutaneous pain-perception and reaction. There were 20 individuals in each group.

subjects was ± 15 per cent, a much smaller variation than our —40 to +50 per cent variation from a mean. This discrepancy may be due to the difference in the technique used to elicit a description of the pain-perception end-point. The fact that individual differences can occur in the threshold of sensory perception of as great magnitude as those we have demonstrated and recorded here is a point, we believe, of major clinical, as well as academic, importance.

In studying the effects of physical and mental fatigue, nervous tension, 48-hour fasting, induced acidosis and alkalosis, and the administration of acetyl-beta-methyl-choline and of epinephrine, variations were considered significant only if the values were 5 per cent or more beyond the normal pain sensitivity variation of a given subject, as determined under certain standard conditions. *Physical fatigue,* produced by a 15-minute fast run or by a one-hour fast walk, caused no change in cutaneous pain-perception or pain-reaction in 10 college undergraduates. On the other hand, *mental fatigue,* from an 8-hour study period, caused a fall in pain-perception of 8 to 10 per cent below the lower limits of normal variation in 3 of the same students, and in 3 others, the pain-perception threshold fell to the lower limits of their established normal variation. The pain-reaction values in these 6 subjects showed a fall parallel with the change in pain-perception. Four subjects showed no change after induced mental fatigue in either pain-perception or pain-reaction.

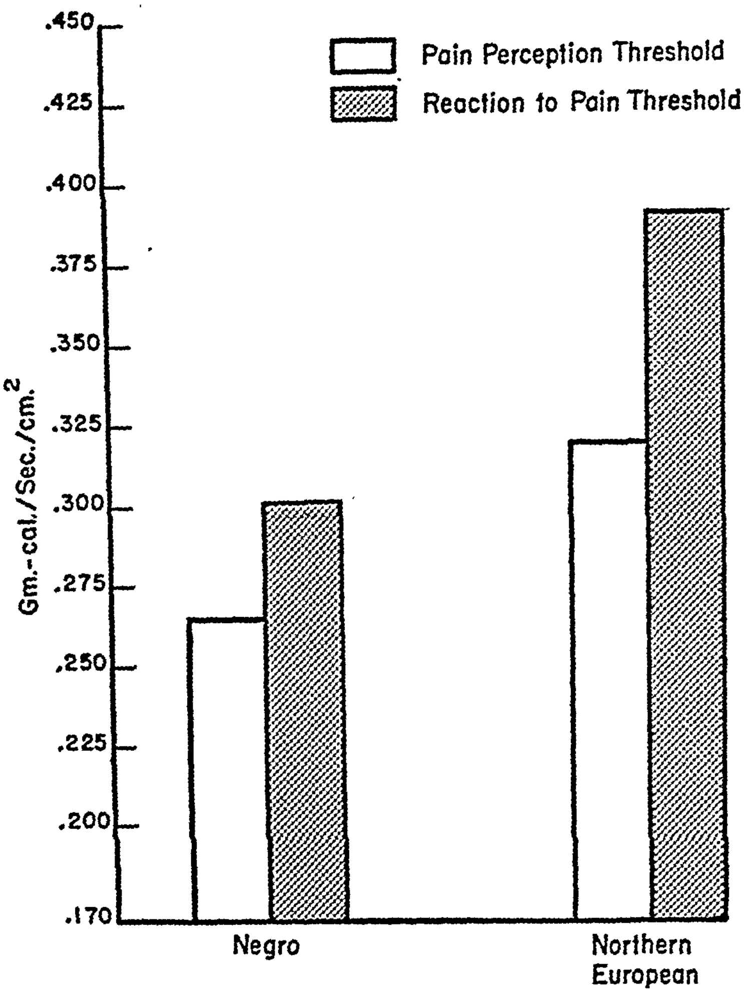

FIG. 4. RELATION OF CUTANEOUS PAIN-PERCEPTION THRESHOLD AND CUTANEOUS PAIN-REACTION THRESHOLD TO RACE

Eighteen subjects in each racial group of same sex and age. Note the greater spread between perception and reaction (wincing) in the Northern European subjects than that observed in a group of Negroes. Each column represents an average of the entire group.

As a test for nervous tension, 12 medical students were studied just before their oral examinations for intern appointments. One subject had a fall of 20 per cent, and 2 others, from 10 to 15 per cent, in both pain-perception and pain-reaction thresholds. Eight of the group showed no change in pain sensitivity. All 12 subjects were retested 2 weeks after hearing the results of their examinations, and in all, the pain threshold measurements were found to have returned to their original levels.

Daily variations in a given individual's pain sensitivity were studied by repeated tests on 15 hospital technicians. On 12 different days, over a period of 3 months, each subject was tested at 9 a.m. and again just before leaving the hospital at 5 p.m. In a majority of instances, pain-perception and reaction values were at the upper limit of their normal variation in the morning and at the lower limit after a day's work. The average percentage variation between the results of the morning and the late afternoon tests was from ± 3 per cent to ± 7 per cent.

Five subjects were tested after the subcutaneous injection of 10 minims of a 1 : 1,000 solution of epinephrine, and again after the subcutaneous injection of 12 mgm. of acetyl-beta-methyl-choline. They were tested also during moderately severe

acidosis and again during severe alkalosis. The influence of a 24-hour fasting period was studied in 4 individuals. None of these procedures caused any significant alteration in either pain-perception or pain-reaction. Attention was also given to the relationship between sex and pain sensitivity. While the average male was found to have slightly higher pain-perception and pain-reaction thresholds, the difference was not sufficiently marked to be of significance.

VISCERAL PAIN SENSITIVITY

Method. The apparatus for measuring visceral sensitivity consisted of a balloon 1½ inches long, to which was attached a U-shaped water manometer. The balloon was introduced through the nose and secured in position 2 to 3 inches above the cardiac end of the esophagus. Air was passed into the balloon by a 50 cc. syringe at a rate of a 2 cm. rise of water pressure per second. The subject was taught to point to the location where a sensation of substernal fullness first occurred. When it was thought that the instructions were understood, 10 observations at 1-minute intervals were made on each subject, the same consideration being given to error in measurement as in the cutaneous pain tests.

Results. The results of the visceral sensory threshold measurements on 29 normal subjects showed values ranging from a level of 15 cm. of water pressure for the most sensitive subject to 89 cm. for the least sensitive (Figure 5). The percentage variations ranged from — 60 per cent to + 58 per cent of the mean average value of 37 cm. of water pressure, with a standard error of 3 cm. For a given subject, tested under standard conditions, the individual percentage variation was from ± 5 per cent to ± 18 per cent. This series was too small for study of visceral sensitivity in relation to age or race. Comparison of the visceral sensory threshold with the cutaneous pain-perception threshold in each case was measured, however, and the rank coefficient correlation of the two measurements for the group of 29 was 0.57 with a probable error of 0.9. Except for one subject who had an extremely high cutaneous pain-perception threshold but a low threshold for visceral perception, these figures indicate a fairly significant correlation of cutaneous and visceral sensitivity for this group.

DISCUSSION

The reliability of the results of any experiments depends upon the recognition of certain variables

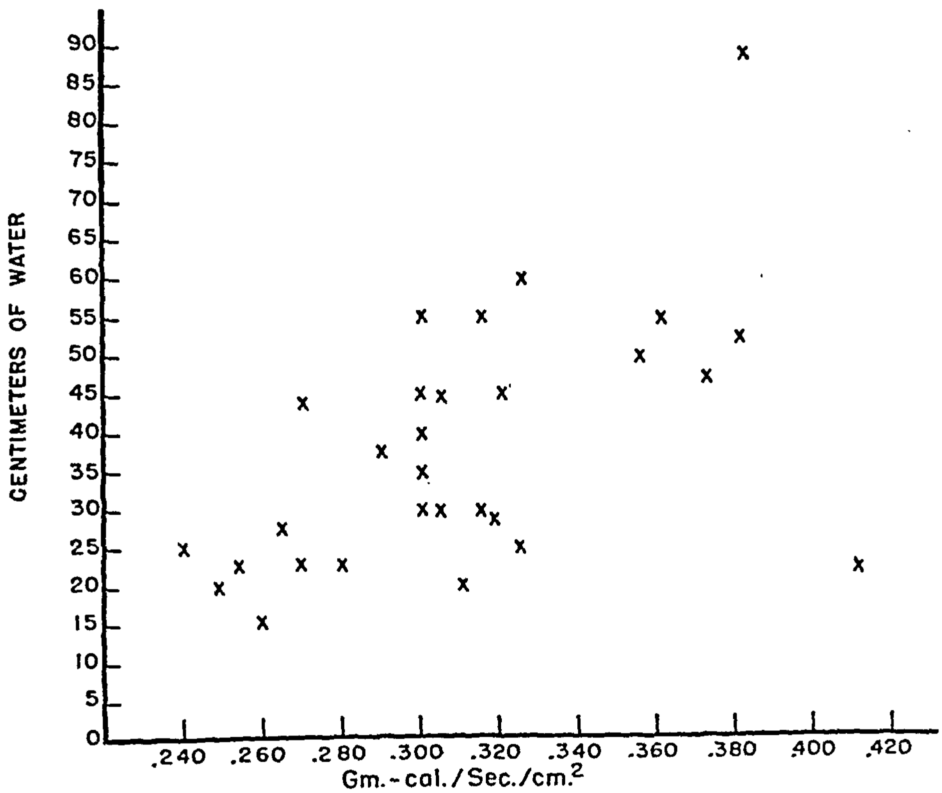

FIG. 5. CORRELATION OF CUTANEOUS PAIN-PERCEPTION THRESHOLD AND VISCERAL SENSORY THRESHOLD

as possible sources of error and the care with which they are controlled. To handle the variables encountered in the measurement of cutaneous and visceral sensitivity, the following questions were applied to the technique employed in the present study. (*1*) Is the stimulus a measurable one? (*2*) Is the stimulus applied to that portion of the body where the neurohistologic variations are at a minimum in different individuals? (*3*) Does the stimulus produce a readily appreciated end-point of pain? (*4*) Is proper consideration given to those factors which at the time of the test may influence the pain sensitivity level? (*5*) Is the proper question technique used to elicit an adequate description of the stimulus?

Cutaneous pain sensitivity

(*1*) The *stimulus* for cutaneous pain, molecular heat (10), was measured directly by a radiometer and expressed as an absolute end-point value of gram cal. per second per $cm.^2$ of skin surface. The radiometer was sufficiently sensitive to record a smaller difference in measurement than could be appreciated physiologically. The line voltage drop afforded a possible source of error, but it was of a smaller magnitude than the physiologic error of interpreting the pain end-point.

(*2*) Its convenient location, the small fluctuations in skin temperature, and the minimal variation in epidermal and in subcutaneous thickness made the midline of the forehead the most desirable *area of stimulation*. No subject showing sunburn, callus, or scars, which might modify the results of the tests (6), was accepted. Whatever variation in thickness of subcutaneous tissue existed was considered of minor importance, because the pain fibers extend as far superficially as the basal layers of the epidermis, and such fibers are held to be the exclusive carriers of cutaneous pain impulses (12). Variations in epidermal thickness, therefore, are of considerable importance, but such variations were found to be small in 6 subjects on whom linear forehead biopsies were taken. The variation in epidermal thickness was not more than $\pm$ 8 per cent in these individuals whereas the pain-perception thresholds varied as much as 30 per cent. We concluded that the small variations in forehead epidermal thickness play a minor role in modifying cutaneous pain determinations.

(*3*) That the heat-radiation stimulus produced a *readily appreciated end-point of pain* was evident from the fact that 88 per cent of all the subjects tested described adequately the pain-perception end-point by the neutral technique alone. The remainder appeared to describe the end-point accurately only after some preliminary discussion as to the sensations ordinarily experienced, or failed to appreciate the pain end-point, either with or without preliminary comments. The last group was not included in the series. The adequacy of the heat stimulus in producing a satisfactory pain end-point may be explained in part by the sudden transition, at the end of the 3-second exposure, from diffuse heat to a distinct sharp prick, and in part by the fact that the sensation was not confused by other modalities, such as touch and pressure, as with mechanical and electric pain-producing instruments. The results of the inquiry as to the resemblance of the sharp jab end-point to the subject's concept of pain were interesting. Ninety per cent of those tested felt that the sensation experienced as the perception end-point was a form of pain, because of its initial hurting quality. No correlation was found between the results of the experimental pain and the subject's appraisal of pain sensitivity. This agreed with the findings of Wolff and his associates.

The pain-reaction end-point was readily observed by watching for the beginning contraction of the eyelids at the outer canthus. It was thought at first that as the subject became accustomed to the stimulus by repeated tests, the reaction level would rise, but this was not the case, except for the first few exposures at or above the pain-perception level. Only a small number of subjects could make any appreciable alteration in their pain-reaction level, even when asked to keep from wincing as long as possible. Furthermore, no appreciable change in the reaction findings resulted from having the subject keep his forehead at the aperture until the end of the 3-second exposure.

It could not be determined which modifying factors were important and should be controlled during the test. Fatigue, apprehension, nervous tension, and medication were all suspected and were excluded so far as possible. The variable that seemed the most important, and the most

difficult to standardize, was the subject's description of the pain-perception end-point. Such descriptions were considered to be of more value when the examiner made no comments prior to the performance of the test. The end-point of pain was described variously as a "sharp jab," "sharp sting," "sharp jab of a wire," or "needle prick." The frequent remarks, "that hurt," "that pained," "that sizzled," or "that branded me" were not accepted as descriptions because they could apply not only to the threshold of pain-perception but to the stimulus levels considerably over the pain-perception threshold. The fact that by our technique, 88 per cent of the subjects agreed upon the distinct end-point quality of the heat-pain stimulus without suggestion, and were consistent in their pain-perception values whenever they were tested, indicates that quantitative measurements of subjective phenomena can be accurately determined, provided the tests are correctly standardized.

Visceral pain sensitivity

The possible errors of measurement for visceral sensory determinations were adequately controlled once a readily appreciated subjective end-point had been determined. That end-point was a beginning awareness of substernal fullness from balloon distention of the esophagus. This was the first substernal sensation experienced from inflation of the balloon and, therefore, was easily appreciated as a distinct quality of sensation. A pain end-point with a definite hurting quality, however, could not be measured. Increasing balloon distention produced in some individuals a feeling of oppression, in others "heartburn," a "cramp ache," a "sharp stab," but no one clear end-point which was agreed upon by all subjects as a beginning pain. In addition, the transition from the beginning substernal fullness to any of these qualities of sensation was so gradual that it was difficult to indicate the exact point of its onset. The measurement of the stimulus was easily controlled, provided the subject had been taught to report the sensation the instant he felt it, and provided the balloon was inflated at a uniform rate of 2 cm. rise of water pressure per second. Any sudden, rapid inflation of the balloon was apt to cause a lower visceral sensory threshold reading.

Individual variation in the calibre or structure of the esophagus could not be ascertained. There was considerable variation at times in the tonicity of the esophageal wall, and this was thought to be a modifying factor until tests were made with the esophagus relaxed by the subcutaneous injection of atropine. Atropine sulphate, grains $1/75$, administered subcutaneously, produced in 4 individuals as much as a 30 per cent drop in intra-esophageal pressure but failed to modify the previously established visceral sensory threshold findings. It is possible that variations in tonicity of the esophagus during any one test were produced by the amount of discomfort and anxiety due to having a tube placed through the nose and to its irritation of the pharynx. Much of the anxiety from this physical inconvenience disappeared before the test had been completed, but the measurements at that time did not vary appreciably from those established at the start of the observations.

Briefly stated, we believe that these measurements of pain sensitivity on 200 normal subjects indicate that there are considerable variations in pain-perception and pain-reaction. The relatively narrow margins within which a given individual's pain-perception and reaction values varied, compared with those for the entire group, make it probable that the differences due to age and race which were found are significant. The relatively high correlation of the visceral sensory threshold with the cutaneous pain-perception threshold is suggestive, but does not prove that an individual who is hypersensitive to one type of pain stimulus will show a corresponding degree of sensitivity to another pain-producing agent. The clinical importance of variations in pain-perception and pain-reaction remains to be ascertained.[4] It may be that, in disease states, reaction to pain is as important as, or more important than, differences in pain-perception. What has been described as pain-perception probably represents a purely sensory phenomenon. What has been described as

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[4] It is highly significant that, in recent studies, the authors have found that the subcutaneous administration of morphine sulphate in ordinary clinical doses produced no greater variations in pain threshold than those noted in this communication. This finding provides obvious proof of the possible clinical significance of such variations.

pain-reaction may well represent a psychologic phenomenon which may assume actual clinical importance.

SUMMARY

1. Two hundred normal subjects, of various races and ages, have been tested for cutaneous pain sensitivity by a modification of the heat-radiation apparatus of Hardy, Wolff, and Goodell. Twenty-nine of this series were tested also for visceral sensitivity by balloon distention of the lower esophagus.

2. Two end-points were measured for cutaneous pain; a beginning sharp jab sensation for the pain-perception threshold; and the first evidence of wincing, as observed at the outer canthus of the eye, for the pain-reaction threshold. The only readily recognizable end-point for initial visceral sensitivity was a sensation of substernal fullness, experienced from balloon distention.

3. Considerable variation was found, both as regards pain-perception and pain-reaction. Pain sensitivity decreased with age. A group of Negroes had a lower pain-perception threshold than a comparable group of Northern Europeans. The Negro also reacted more readily than the Northern European to the pain stimulus, as evidenced by the narrow spread between his pain-perception threshold and the level at which wincing occurred. The Mediterranean races tested had both pain-perception and pain-reaction values which corresponded closely with those of the Negro.

4. Of a number of possible modifying factors, such as the administration of epinephrine or acetyl-beta-methyl-choline, severe acidosis and alkalosis, mental and physical fatigue, nervous tension, and 48-hour fasting, only mental fatigue and nervous tension produced any significant changes in cutaneous pain sensitivity.

5. The possible sources of error in measurement of cutaneous and visceral sensitivity are enumerated and discussed.

6. It is believed that what has been described as pain-perception probably represents a purely sensory phenomenon. Pain-reaction may well represent a psychologic phenomenon which may assume actual clinical importance as, for example, in a group of neurotic subjects.

7. The magnitude of individual threshold variations in pain-perception and reaction, encountered in these studies on normal subjects, is of real clinical significance.

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TRANSFERS OF INTRACELLULAR POTASSIUM IN EXPERIMENTAL DEHYDRATION [1]

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Prolonged dehydration and starvation in the dog result in the sacrifice by the organism of more potassium than that derived from the breakdown of tissue (1). There is no comparable sacrifice of sodium. As a result, the diminution of intracellular fluid is exaggerated while that of extracellular fluid is minimized. The present experiments seek to define more generally the circumstances under which this loss of intracellular potassium may occur. This has been done by studying the transfers of potassium and of nitrogen associated with various alterations in the amount and distribution of water and of salts within the body.

METHODS AND CALCULATIONS

The methods of chemical analyses and of calculating the changes in the distribution of water and electrolytes have been described in detail elsewhere (1). The essential formulae used are reproduced here without rationale.

(*a*) Total water change, ΔW:

In acute experiments, $\Delta W_I = \Delta Wt'$. (*1*)

In chronic experiments (12 hours or longer), (*2*)

$$\Delta W_I = \Delta Wt' + 0.49P + \frac{\text{Total calories} - 4.1P}{9.3}$$

where

ΔW_I = total water change in experiments where a metabolic calculation could be made,

$\Delta Wt'$ = the weight change corrected for solids lost,

P = the protein burned, obtained by multiplying the nitrogen balance, corrected for changes in NPN, by 6.25,

Total calories = 2 per kilogram body weight per hour, decreasing 1.5 per cent per day.

In the chronic experiments where a metabolic calculation could not be made:

$$\Delta W_{II} = \Delta E_{Cl} + \Delta I_{II} \quad (3)$$

(*b*) Extracellular volume change, ΔE:

$$\Delta E = E_2 - E_1, \quad (4)$$

E_2 being calculated in two independent ways:

$$E_{Cl_2} = \frac{E_1\,Cl_1 + b_{Cl}}{Cl_2}, \quad (5)$$

$$E_{Na_2} = \frac{E_1\,Na_1 + b_{Na}}{Na_2}, \quad (6)$$

where

b_{Cl} and b_{Na} = balance of chloride and of sodium, respectively.

E_1 = initial extracellular fluid = liters corresponding to one-fourth of the body weight in kilograms.

Cl_1 and Cl_2 = initial and final concentration of chloride in extracellular water.

Na_1 and Na_2 = initial and final concentration of sodium in extracellular water.

The concentrations of chloride, sodium, and potassium in extracellular water (ECW) were calculated from the serum concentrations (s) by the use of a Donnan factor of 0.95:

$$Cl_{ECW} = Cl_s/W_s \times 0.95 \quad (7)$$

$$Na_{ECW} = Na_s \times 0.95/W_s \quad (8)$$

$$K_{ECW} = K_s \times 0.95/W_s \quad (9)$$

Where W_s = grams of water in one ml. of serum. In the chronic experiments (Experiments 11C, 13A, and 14), W_s was assumed equal to 0.93 grams per ml. serum; in the acute experiments it was determined chemically.

(*c*) Intracellular volume change, ΔI:

This was estimated in two independent ways.

$$\Delta I_I \text{ (first method)} = \Delta W - \Delta E_{Cl}, \quad (10)$$

$$\Delta I_{II} \text{ (second method)} = 2.7\,P + \frac{b_K' - \Delta B(0.65\,Wt. - E_1)}{B_2} \quad (11)$$

where

$Wt.$ = initial body weight in kilograms,

P = protein burned, in kilograms,

$b_K' = b_K - (K_{ECW_2} \times E_2) + (K_{ECW_1} \times E_1) - 380\,P$ = balance of "excess" potassium, (*12*)

b_K = total balance of potassium,

$$\Delta B = B_2 - B_1, \quad (13)$$

$$B = Na_{ECW} + 10 \text{ m.eq. per liter.} \quad (14)$$

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[2] National Research Council Fellow in the Medical Sciences, 1940–42.

The term "balance" or the symbol "b" indicates a retention by the organism when the sign is positive and a loss when the sign is negative. "Change" (Δ) likewise indicates a gain or loss according to the sign.

(*d*) Balance of "excess potassium," b_K':

The term "excess potassium" is used to designate potassium which moves in excess of nitrogen, *i.e.*, potassium, leaving or entering the intracellular fluid, which is not associated with the catabolism or anabolism of protein. It is the sum of two elements, one already excreted in the urine and one which has moved into the extracellular fluid but has not yet been excreted, and is, therefore, a balance in relation to the cells rather than the organism. It is given by Equation 12. The factor by which P is multiplied, 380, is based on the assumption that the normal K : N ratio in dog muscle is 2.38 m.eq. of potassium per gram of nitrogen. This represents the average ratio of potassium to nitrogen found in the intracellular phase of skeletal muscle of 20 normal dogs by Hastings (2), assuming 92.6 per cent of the solids to be protein (3).

This K : N ratio is the lowest ratio in 6 series of analyses of skeletal muscle in dogs (2 to 7), the highest, 2.94, being that found by Eichelberger. Use of the lowest value results in somewhat higher values for "excess" potassium, but the difference is relatively slight. Had Eichelberger's ratio, rather than that of Hastings, been used in the control periods of the 3 balance experiments, the mean balance of "excess" potassium (b_K') would have changed from −0.17 to −0.13 m.eq. per kilogram body weight. This difference is statistically negligible, since the standard deviation of this mean was ±0.31 m.eq. per kilogram.

In a starving dog, allowed to drink water *ad libitum* (unpublished experiment), the ratio of the negative balance of potassium to that of nitrogen, from the sixth to the fourteenth day of the fast, was found to be 2.30. This should approximately represent the ratio of these substances in wasting tissue, and is in good agreement with the ratio of 2.38 derived from Hasting's muscle analyses.

EXPERIMENTAL PROCEDURE

Adult female dogs were used throughout. Net balances of water, chloride, potassium, and nitrogen were measured in all experiments. In the 3 *chronic* balance experiments (11C, 13A, and 14), dogs were fed a constant daily diet of "Maro" Meat Mixture for 8 to 13 days. One kilogram of this meat mixture contained by analysis: water, 736 grams; chloride, 117 m.eq.; potassium, 66.2 m.eq.; and nitrogen, 23.1 grams. In Experiment 11C, the daily intake of the meat mixture was 300 grams, in Experiment 14, 250 grams, and in Experiment 13A, 400 grams (except on the 5th day when the intake was 280 grams). Water *ad libitum* was supplied. Balances were determined for each daily period. On certain days, sodium chloride in hypertonic, isotonic, or hypotonic solution was given intravenously. The balances of total potassium, nitrogen, and of excess potassium on these days of saline injection were compared with mean control values. In computing the mean control values, all remaining daily periods were used, except the 2 daily periods following each period of significant "excess" potassium loss, subsequent to a saline injection; 14 periods in all were included.

The 13 acute experiments lasted from 2 to 24 hours. Sodium and chloride balances were followed in all of the experiments. Preliminary ureteral ligation, under dial anesthesia, was done in 4 experiments (15 to 18) in which 5 per cent sodium chloride was injected. Two of the animals (Experiments 15, 16) received the injection intravenously, 2 (17, 18), intraperitoneally. Similar intraperitoneal injections of 5 per cent saline were given to 4 other dogs (Experiments 19, 20, 21, 11E) with intact kidneys, the first 3 under dial anesthesia and the last under morphine analgesia. In the 6 dogs receiving intraperitoneal injections (Experiments 17, 18, 19, 20, 21, 11E), a volume of peritoneal fluid, somewhat greater than that injected, was withdrawn 30 or 40 minutes after the initial injection. Since the concentration of salt in this fluid was always much less than 5 per cent, the final effect of the combined intraperitoneal injection and withdrawal was an increase in the salt content and a decrease in the water content of the body in all 6 experiments. In still another experiment (13C), a solution of 5 per cent glucose in 10 per cent urea was injected intraperitoneally, and 4 hours later an equal volume of peritoneal fluid was removed. This fluid now contained salt but had a lower urea concentration than did the original solution. The absorbed urea provoked a diuresis so that the procedure resulted in a water loss without hypertonicity of the body fluids, at least for the first 12 hours. In still another group of 4 experiments with diuresis, unanesthetized dogs were each given intravenously one of the following solutions: 5 per cent glucose in 10 per cent urea (12C); 1.1 per cent sodium sulfate in 10 per cent urea (13B); 5 per cent glucose alone (11D); and 1.1 per cent sodium sulfate alone (22A).

RESULTS

(*a*) *Chronic balance experiments.* The results of these 3 experiments are presented in Table I. The range of daily variations of total water (ΔW) did not exceed ±4 per cent. Serum potassium concentration was virtually unchanged from day to day. The mean balance of excess potassium, b_K', in 14 normal daily periods in the 3 dogs, equalled −0.17 m.eq. per kilogram of body weight. Changes in b_K' in the periods of saline injection were considered significant only if they differed from this mean value by an amount exceeding twice the standard deviation, *i.e.*, if they were greater than +0.45 or less than −0.79 m.eq. per kilogram.

The injection of hypertonic (5 per cent) sodium chloride solution regularly produced a significant negative balance of excess potassium. The

TABLE I

Chronic experiments: analytical data, and calculations of balance of "excess" potassium and of changes in body fluid phases

Experiment	Intravenous NaCl solution conc.	Intravenous NaCl solution amt.	Time from start of experiment	Body weight	Blood concentration NPN	Serum concentration Na	Serum concentration Cl	Serum concentration K	Balance Cl	Balance K	Balance N	b_K'*	ΔE_{Cl}†	ΔI_{II}‡	ΔW_{II}§
	per cent	cc.	days	kgm.	mgm. per cent	m.eq. per liter	m.eq. per liter	m.eq. per liter	m.eq.	m.eq.	grams	m.eq. per kgm.	liters	liters	liters
11C			0	11.68		142.6	113.0	4.83							
	None		1	11.72	35	142.6	111.5	4.49	+ 2.9	− 0.1	−0.4	+0.14	+0.06	±0	+0.06
	1.8	500	2	11.48	35	143.3	115.0	4.12	+ 3.8	−16.2	−3.0	−0.65	−0.06	−0.10	−0.16
	None		3	11.58		142.3	110.3	4.61	+ 1.1	+ 8.3	+0.8	+0.48	+0.14	+0.07	+0.21
	None		4	11.40	46	141.5	111.9	4.74	−22.0	−10.5	−3.8	+0.11	−0.22	−0.07	−0.29
	5.0	300	5	10.98	40	146.0	118.1	4.60	+29.0	−28.0	−2.5	−2.03	+0.07	−0.31	−0.24
	None		6	11.36	30	142.5	105.8	4.38	−12.7	+ 8.3	−1.3	+0.61	+0.23	+0.13	+0.36
	None		7	11.36	36	142.5	108.0	4.87	+12.3	+ 6.0	−2.1	+0.94	+0.04	+0.03	+0.07
	0.3	500	8	11.40	37	139.2	108.7	4.44	+11.8	− 5.1	−2.3	+0.15	+0.07	+0.10	+0.17
	None		9	11.34	33	140.9	110.0	5.16	− 0.5	− 3.7	−0.5	−0.47	−0.05	−0.10	−0.15
	None		10	10.92			105.1	4.43	−41.6	−21.9	−8.4	+0.02	−0.20		
	None		11	10.78			114.0	4.23	− 8.4	− 0.7	−1.3	+0.38	−0.29		
	None		12	10.88			112.4	4.16	+ 7.7	+ 6.6	+2.6	+0.02	+0.10		
	None		13	10.84			110.9	4.61	+ 7.7	+ 1.3	+0.3	−0.10	+0.10		
13A			0	11.66	24	134.0	108.1	4.51							
	None		1	11.60	23	136.5	106.5	4.74	+ 0.1	+ 9.2	+4.8	−0.29	+0.05	−0.03	+0.02
	5.0	350	2‖		24	160.6	130.2	4.57	+57.4	−31.2	−3.9	−1.76	−0.16	−0.83	−0.99
			2‖	10.64	25	147.5	119.9	4.27	−64.6	− 4.0	+3.5	−0.89	−0.22	+0.31	+0.09
	None		3	11.46	26	133.8	109.5	4.62	−11.0	+ 8.7	±0	+0.69	+0.15	+0.44	+0.59
	None		4	11.50	24	145.0	106.2	3.93	−26.4	+11.5	+0.5	+1.06	−0.13	−0.23	−0.36
	0.9	500	5	11.32	26	139.9	106.9	4.38	+ 7.4	− 4.2	−0.3	−0.37	+0.04	+0.13	+0.17
	None		6	11.28	23	143.5	101.8	3.60	− 2.1	+ 9.3	+3.9	+0.12	+0.11	−0.06	+0.05
	None		7	11.32	25	141.6	106.3	4.76	− 8.5	+ 7.0	+2.7	−0.11	+0.19	+0.12	−0.07
	None		8	11.30	24	139.9	105.3	4.25	− 1.5	− 0.5	+2.9	−0.57	+0.02	+0.08	+0.10
14			0	7.36	30	138.8	108.7	4.89							
	None		1	7.30	26	138.3	111.8	4.56	− 5.4	− 0.2	−0.6	+0.24	−0.09	+0.02	−0.07
	5.0	200	2‖		25	153.8	120.7	4.55	+33.7	−16.6	−1.8	−1.77	+0.12	−0.39	−0.27
			2‖	6.82	28	150.6	119.0	4.35	+ 0.6	+ 6.5	+3.9	−0.30	+0.03	+0.10	+0.13
	None		3	7.82	28	138.3	109.2	3.60	+ 5.1	+11.1	±0	+1.73	+0.20	+0.31	+0.51
	None		4	7.42	24	143.6	106.6	4.33	+11.7	+13.6	+4.2	+0.12	+0.16	−0.04	+0.12
	0.9	350	5	7.40	30	141.7	106.6	4.35	+ 2.1	+ 4.9	+2.5	−0.05	+0.01	+0.08	+0.09
	None		6	7.26	25	140.4	102.1	3.91	−22.4	− 1.3	−0.7	+0.18	−0.09	+0.02	−0.07
	None		7	7.28	22	144.6	109.3	4.70	+ 7.1	+ 4.0	+1.5	−0.17	−0.08	−0.06	−0.14
	None		8	7.28	24	141.1	107.2	4.77	+ 4.4	+ 4.5	+1.4	+0.15	+0.07	+0.11	+0.18

* b_K' = balance of "excess" potassium (see Equation 12 in METHODS AND CALCULATIONS), expressed as m.eq. per kilogram of initial body weight.

† ΔE_{Cl} = change in extracellular water volume (Equations 4, 5).

‡ ΔI_{II} = change in intracellular water volume (Equation 11).

§ ΔW_{II} = change in total water volume (Equation 3).

‖ Balances on the 2nd day were determined at the end of 3 hours after the saline infusion and again at the end of 24 hours, and are given in that order.

In all tables time from start of experiment indicates end of period, at which time serum analyses were made and balances determined. Quantities are expressed per individual period rather than cumulatively. In designation of experiment, number refers to the individual dog; where a dog was used more than once, letter refers to successive experiments.

greater part of the excess potassium was excreted within the first 3 hours after the infusion (Experiments 13A, 14). Isotonic (0.9 per cent) and hypotonic (0.3 per cent) solutions evoked no such response. The response to mildly hypertonic solutions (1.8 per cent) was equivocal. In the 48 hours following hypertonic injections, there was a marked decrease in the potassium excretion, so that b_K' now became significantly positive. This continued until a considerable part of that lost following the infusion had been restored.

(*b*) *Acute experiments.* The results of these experiments appear in Tables II and III. In the 4 dogs with ligated ureters (Experiments 15, 16, 17, 18), the injection of hypertonic saline either intravenously or intraperitoneally was without significant effect on the potassium distribution.

TABLE II

Acute experiments: exchanges of water, electrolytes, and nitrogen

Experiment	Solution injected	Time from start of experiment	Net intake*					Urinary output				
			H_2O	Na	Cl	K	N	H_2O	Na	Cl	K	N
		hours	*cc.*	*m.eq.*	*m.eq.*	*m.eq.*	*grams*	*cc.*	*m.eq.*	*m.eq.*	*m.eq.*	*grams*
15§	5 per cent NaCl†	4.2	239	246.8	248.8							
16§	5 per cent NaCl†	3.2	250	214.0	214.0							
17§	5 per cent NaCl‡	2.0	−265	67.8	74.9	−1.5	−0.14					
18§	5 per cent NaCl‡	3.2	−208	77.9	84.3	−1.5	−0.15					
19	5 per cent NaCl‡	10.5	−149	77.8	83.6	−1.4	−0.07	20	3.7	4.7	2.6	0.28
20	5 per cent NaCl‡	5.3	−302	166.7	177.0	−2.2	−0.22	12	1.6	1.4	0.8	0.31
21	5 per cent NaCl‡	3.2	−213	123.3	132.2	−2.4	−0.13	37	10.9	13.2	1.7	0.63
		22.0	− 14	− 2.4	− 2.2			95	28.0	29.3	11.0	1.60
11E	5 per cent NaCl‡	3.5	− 85	265.0	279.4	−2.2	−0.12	117	42.9	40.8	4.7	0.83
		25.3	− 20	− 3.5	− 2.6			415	187.0	164.3	26.0	3.55
13C	5 per cent glucose +10 per cent urea‡	11.8	0	−72.0	−58.5	−2.0	25.41	390	4.8	6.5	13.6	7.52
		23.7	− 16	− 2.1	− 1.8			595	1.7	4.3	28.5	15.80
12C	5 per cent glucose +10 per cent urea†	22.7	560				26.10	1405	28.1	37.2	32.6	31.10
13B	10 per cent urea +1.1 per cent Na_2SO_4†	21.8	550	113.0			25.60	1970	142.7	60.3	54.6	28.50
11D	5 per cent glucose†	23.0	610					705	2.3	16.4	15.8	5.50
22A	1.1 per cent Na_2SO_4†	21.8	725	149.0				690	154.4	38.7	23.2	5.10

* Intake corrected for loss in serum specimen taken for analysis and peritoneal fluid withdrawn.
† Injected intravenously at start of experiment.
‡ Injected intraperitoneally at start of experiment.
§ Ureters tied.

In 3 of the 4 dogs (19, 21, 11E) with intact kidneys, on the other hand, intraperitoneal injection of 5 per cent saline with subsequent withdrawal of fluid was followed by a significant loss of excess potassium in the urine. This negative balance developed only gradually (Experiment 21). This may account for the single exception (Experiment 20), since this dog died within 6 hours, before much urine had been passed. It was already very clear at the end of 11 hours (Experiment 19), and was still more evident after 24 hours. The loss of potassium was less rapid than that which followed intravenous injection of the same solution in the chronic balance experiments, and was associated with less acute diuresis. Concentration of potassium in serum rose slightly above the initial level in all but 1 experiment (Experiment 18), but, with the possible exception of Experiment 19, all the changes fell within the range of normal variation.

In Experiment 13C, a severe demand for water was coupled with a depletion of extracellular base. The result was a marked excretion of excess potassium even while the sodium concentration was depressed below normal. The serum potassium concentration rose slightly.

Of the last 4 experiments (12C, 13B, 11D, and 22A), the 2 dogs receiving the urea had a much more copious diuresis than did the control animals, and experienced a considerable net dehydration. The control pair merely excreted about enough urine to restore their water content to the initial value. The animals receiving the urea had a large excretion of excess potassium, while the other pair did not. Serum potassium rose slightly in one of the urea experiments but not in the other, while it decreased in the 2 control experiments.

DISCUSSION

The excretion of intracellular potassium in excess of that derived from the breakdown of tissue may occur in a variety of conditions involving alterations of the water or the salt content of the body. Benedict (8) and Gamble, Ross, and Tisdall (9) noted this phenomenon during the initial stages of starvation without water deprivation. Others have observed it in a variety of clinical conditions, such as diabetic coma (10), diarrhea of infants (11), hemorrhage (12), and water deprivation (13). It has been described in experiments with water deprivation in the rabbit (14).

In man, it follows the ingestion of dry sodium chloride (15); in the dog, it follows the intravenous infusion of hypertonic sodium sulfate (16) and the injection of hypertonic saline in animals previously depleted of sodium and chloride (17).

Our experiments reported here and elsewhere (1, 18, 19) give some negative and some positive clues to the factors common to all the diverse states in which there may be a loss of excess potassium. It cannot simply be the result of a temporary increase of plasma volume, since it is absent following intravenous injections of isotonic and hypotonic solutions (Table I, Experiments 11C, 13A, 14) and since it appears as readily after intraperitoneal as after intravenous injections of hypertonic salt solutions (Tables II and III). It may occur with hypertonic sodium sulfate solution (16) and with concentrated urea solution (Tables II, III, Experiments 13C, 12C), as well as with hypertonic sodium chloride infusion (Table I), so that it is not a specific effect of some one salt. It occurs during dehydration without any additional salt being injected (1). Although usually associated with an increased concentration of electrolyte in the body, it may occur without any such increase (Experiment 13C).

In Figure 1, the balance of excess potassium, b_K', is compared with the associated change in base concentration, ΔB, with the change in extracellular water, ΔE, with the change in intracellular water, ΔI, and with the change in total water,

TABLE III

Acute experiments: analytical data, and calculation of balance of "excess" potassium and of changes in body fluid phases

Experiment	Solution injected	Time from start of experiment	Body** weight	Blood conc. NPN	Serum concentration				b_K'	ΔW_I*	ΔE_{Cl}	ΔE_{Na}‖	ΔI_I#	ΔI_{II}
					H_2O	Na	Cl	K						
		hours	*kgm.*	*mgm. per cent*	*grams per liter*	*m.eq. per liter*	*m.eq. per liter*	*m.eq. per liter*	*m.eq. per kgm.*	*liters*	*liters*	*liters*	*liters*	*liters*
15§	5 per cent NaCl†	0	7.70	50	946	146.2	102.7	4.21						
		4.2		69	962	192.0	167.3	4.38	−0.03		+0.63	+0.87		−0.68
16§	5 per cent NaCl†	0	6.16	50	940	144.7	101.8	4.99						
		3.2	6.30	67	957	183.2	161.6	5.75	−0.47	+0.14	+0.62	+0.84	−0.48	−0.49
17§	5 per cent NaCl‡	0	6.70	47	947	135.9	101.7	4.44						
		2.0	6.38	58	952	164.0	134.5	4.96	−0.24	−0.32	+0.12	+0.15	−0.44	−0.44
18§	5 per cent NaCl‡	0	5.50	51	940	143.2	102.6	5.13						
		3.2	5.22	66	941	165.9	143.1	4.74	+0.02	−0.28	+0.14	+0.28	−0.42	−0.29
19	5 per cent NaCl‡	0	4.70	34	948	146.4	110.2	4.58						
		10.5		60	950	171.6	145.2	6.14	−1.11		+0.20	+0.27		−0.31
20	5 per cent NaCl‡	0	9.35	46	940	150.8	114.0	3.99						
		5.3		58	941	181.2	160.6	5.11	−0.45		+0.30	+0.51		−0.64
21	5 per cent NaCl‡	0	7.95	42	947	141.2	110.9	3.72						
		3.2	7.58	38	946	173.3	155.5	3.75	−0.47	−0.37	+0.22	+0.35	−0.59	−0.65
		22.0	7.28	27	948	162.4	146.4	4.86	−1.30	−0.26	−0.07	−0.05	−0.19	+0.10
11E	5 per cent NaCl‡	0	12.22	31	942	139.5	107.2	4.03						
		3.5	11.91	31	941	175.8	149.5	4.60	−0.74	−0.31	+0.56	+0.62	−0.87	−1.01
		25.3	11.23	25	937	156.5	130.2	4.44	−1.22	−0.62	−0.62	−0.77	±0	+0.27
13C	5 per cent glucose	0	11.34	32	938	142.1	106.2	4.82						
	+10 per cent urea‡	11.8	10.64	279	919	130.3	92.6	5.88	−1.77	−0.66	−0.27	−0.45	−0.39	+0.16
		23.7	9.88	126	922	150.5	110.2	5.32	−0.78	−0.72	−0.45	−0.28	−0.27	−0.73
12C	5 per cent glucose	0	12.90	29	936	143.8	104.4	3.76						
	+10 per cent urea†	22.7	11.79	46	922	150.6	111.1	3.86	−1.26	−1.11	−0.53	−0.37	−0.58	−0.49
13B	10 per cent urea	0	11.56	28	947	145.8	101.6	4.16						
	+1.1 per cent Na_2SO_4†	21.8	9.85	34	920	162.0	114.9	4.59	−3.82	−1.71	−0.87	−0.54	−0.84	−0.86
11D	5 per cent glucose†	0	12.22	28	939	146.8	103.5	4.35						
		23.0	12.04	24	942	138.1	105.8	4.00	−0.11	−0.18	−0.20	+0.19	+0.02	+0.23
22A	1.1 per cent Na_2SO_4†	0	15.58	34	939	144.8	103.4	4.20						
		21.8	14.83	31	945	146.2	108.2	3.98	−0.57	−0.75	−0.47	−0.04	−0.28	−0.14

§†‡ See footnotes to Table II.
* ΔW_I = change in total water volume (Equations 1, 2).
‖ ΔE_{Na} = change in extracellular water volume (Equations 4, 6).
\# ΔI_I = change in intracellular water volume (Equation 10).
For other symbols see footnotes to Table I.
** Weights corrected for solids lost.

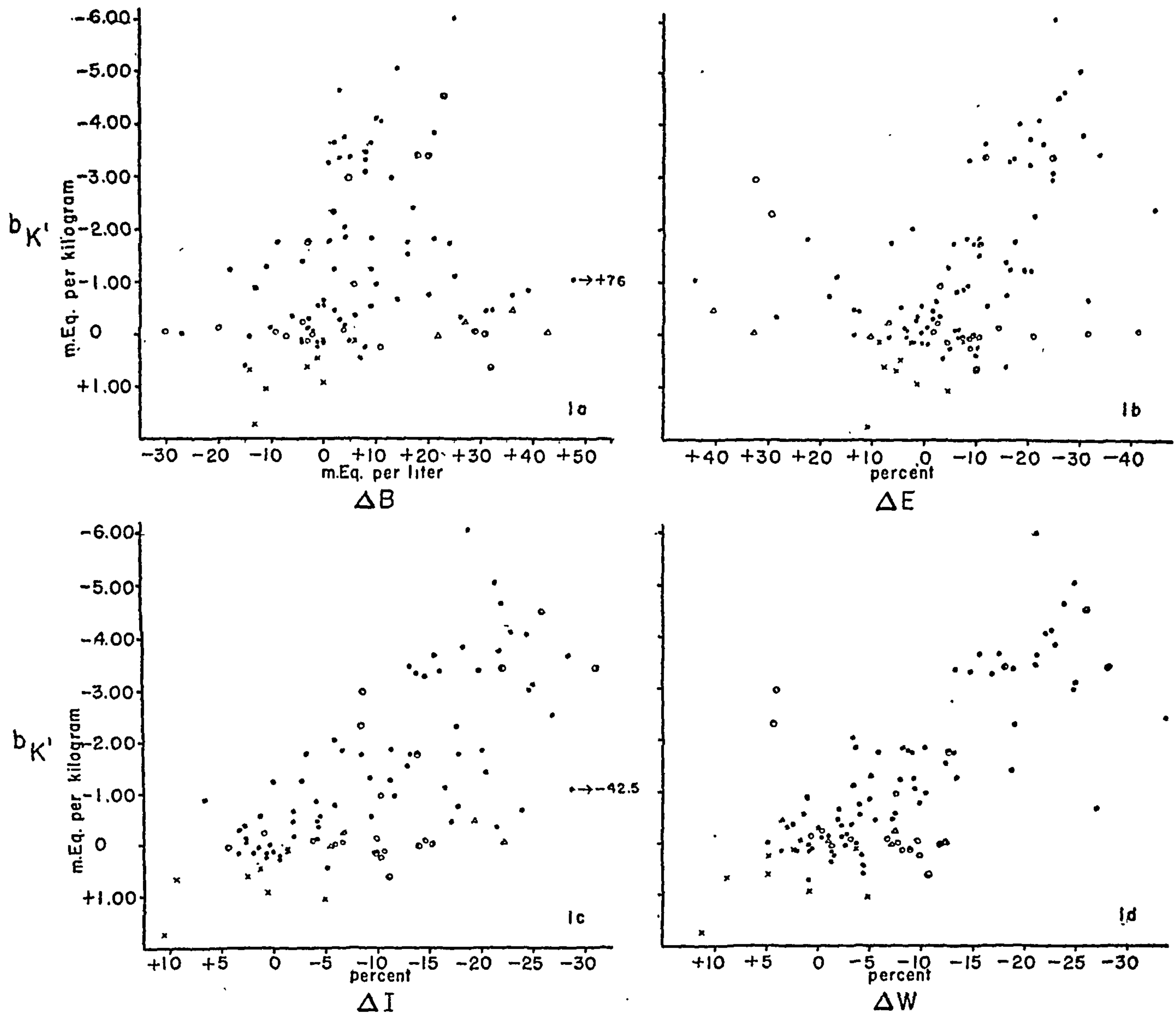

FIG. 1. COMPARISON OF THE BALANCE OF "EXCESS" POTASSIUM, b_K', WITH CHANGES IN TONICITY OF BODY FLUIDS, ΔB (*a*); WITH CHANGES IN EXTRACELLULAR FLUID VOLUME, ΔE (*b*); WITH CHANGES IN INTRACELLULAR FLUID VOLUME, ΔI (*c*); AND WITH CHANGES IN TOTAL WATER VOLUME, ΔW (*d*)

b_K' is plotted along the ordinates in a negative direction, while ΔB, ΔE, ΔI, and ΔW, respectively, are plotted along the abscissae. *Symbols represent balances determined under the following conditions: ureters tied, triangles; during* 48 hours after significant losses of excess potassium in fed dogs, crosses; sodium chloride depletion, open circles; injection of hypertonic sodium chloride solution following sodium chloride depletion, circles containing dots; 2 successive periods during severe water depletion, divided circles; unspecified, solid black circles.

The data are from balances determined for 103 periods, in 24 different dogs, under 20 different experimental conditions producing alterations in water and salt contents of the body.

ΔW. Included are data from 103 different periods, in 24 different dogs, under 20 different experimental conditions, drawn from the experiments reported here and in other papers in our series (1, 18, 19), as well as from unpublished data. There is obviously little significant correlation between b_K' and ΔB or ΔE. There is a rough correlation between b_K' and ΔI, which is not surprising since loss of excess potassium from the cell necessarily favors a reduction in intracellular water. There may be an even better correlation between b_K' and ΔW. These two poor correlations and two good ones indicate that one feature, depletion of the body of water, is common to all these different procedures which result in the excretion of excess potassium.

There are, however, certain important exceptions to the positive correlation between b_K' and ΔW in Figure 1d. During periods of sodium chloride depletion, the loss of excess potassium was small in proportion to the severity of the water loss (open circles in lower right). When hypertonic saline was then injected following two such periods, there was a large loss of excess potassium in the presence of a positive balance of water (circles containing dots in upper left). These exceptions suggest that, while an elevated base concentration is not essential to the loss of excess potassium, it nevertheless favors this movement, while a low base concentration tends to inhibit it. It is also evident that, with continued dehydration, the loss of excess potassium tends to diminish. In the experiments in which balances were measured for two successive periods during water depletion, the loss of excess potassium was uniformly lower, in proportion to the water loss, in the second period than in the first (Figure 1d, divided circles).

Under some circumstances sodium may apparently enter cells in appreciable amounts. It is possible that sodium enters cells in exchange for the potassium which is lost, *i.e.*, these transfers of potassium may merely represent an interchange between intracellular and extracellular base. No water exchange would accompany such an exchange. The balance of excess intracellular sodium, b_{Na}', may be calculated in a manner exactly analogous to that by which the balance of excess potassium is calculated. The final formula is:

$$b_{Na}' = b_{Na} - b_{Na_P} - b_{Na_E} = b_{Na} - 30 \times P - (Na_{ECW_2} \times E_2 - Na_{ECW_1} \times E_1)$$

These various symbols are defined elsewhere (1). In Figure 2, b_K' is plotted against b_{Na}', calculated in all experiments from which sufficient data are available. The poor correlation is evident. In certain cases, intracellular sodium and intracellular potassium do move in opposite directions, so that some measure of exchange does take place. However, these movements are too inconstant and unequal to provide any consistent explanation for the loss of excess potassium.

Great resistance of the concentration of serum potassium to change is apparent in our experiments. When the ureters were tied, there was no appreciable transfer of potassium to the extracellular fluid and no rise in serum concentrations. This is perhaps fortunate, as concentrations not much above normal are toxic (20). When the

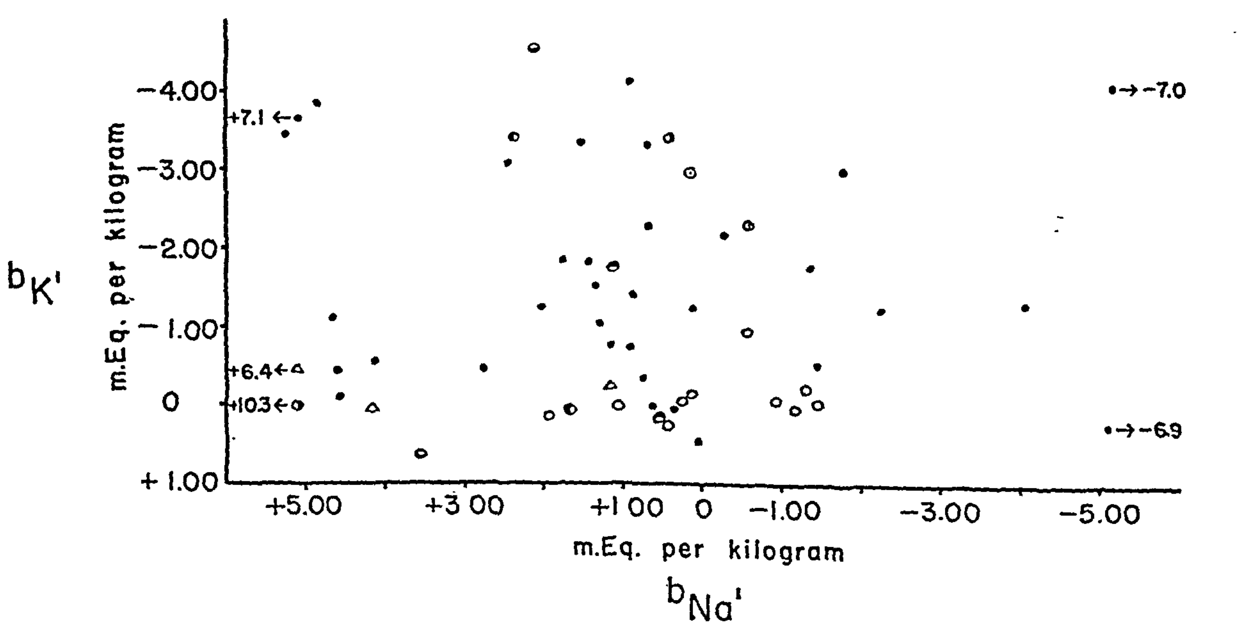

FIG. 2. TRANSFERS OF INTRACELLULAR SODIUM (b_{Na}') AND SIMULTANEOUS TRANSFERS OF INTRACELLULAR POTASSIUM (b_K')

Symbols are as in Figure 1. There is little correlation.

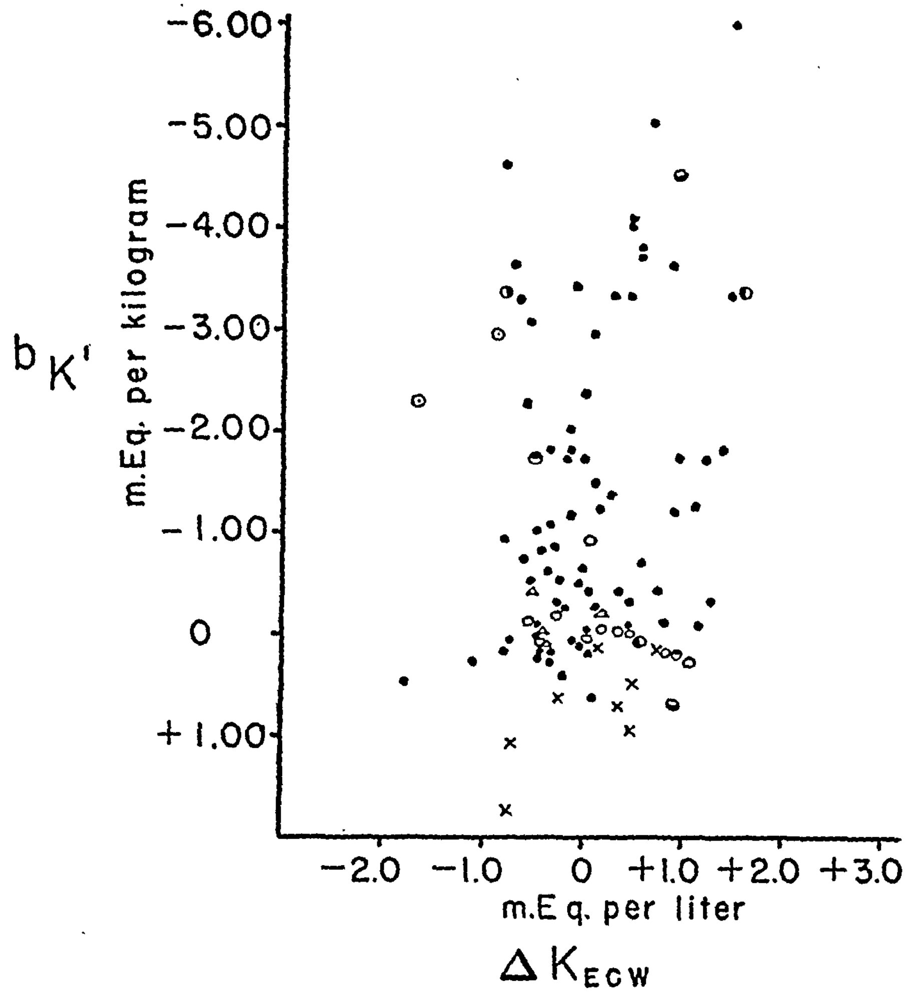

FIG. 3. COMPARISON OF THE BALANCE OF "EXCESS" POTASSIUM, b_K', WITH CHANGES IN THE POTASSIUM CONCENTRATION IN EXTRACELLULAR WATER, ΔK_{ECW}

b_K' is plotted along the ordinate in a negative direction, while ΔK_{ECW} is plotted along the abscissa. Data are from the same periods as those in Figure 1. Symbols are as in Figure 1. Changes in serum potassium concentration associated with anoxia (blood drawn during heart block or after respirations had stopped) are omitted.

kidneys were intact, large amounts of excess potassium were removed from the cells without any consistent or large change in extracellular fluid concentration of potassium (Figure 3). This transfer may have involved alterations in the equilibrium between cellular and extracellular potassium, too small to be detected by present analytical methods. An entire series of reactions must be disturbed in the complex process of transferring potassium from tissue cells to urine. Our data do not clearly indicate which equilibrium is the first to be upset.

Large alterations in the cellular to extracellular potassium equilibrium apparently occur only where cell metabolism is affected, as in anoxia (21). Increases in the concentration of serum potassium, occurring in hemorrhage and intestinal obstruction, have been interpreted as replacements of lost extracellular by intracellular fluid (22 to 25). Such rises in concentration of serum potassium more probably reflect changes in cellular metabolism, since our experiments in uncomplicated water depletion indicate the marked stability of extracellular fluid concentration of potassium, even during extensive transfer and excretion of intracellular water and base.

There is much evidence that dehydration involving the loss of cellular water is less likely to provoke circulatory collapse than dehydration of the extracellular fluid alone (17, 26, 19). Whatever the teleological interpretation, our experiments indicate that the release of potassium from

the intact cell and its renal excretion is a general and reversible physiological response of the organism to severe depletion of water.

CONCLUSIONS

Loss of intracellular potassium in excess of that associated with protein catabolism is a general response to water depletion from any cause. Loss of intracellular water accompanies this loss of excess potassium, with the result that the loss of extracellular water is minimized. Renal activity is essential to effect this loss of potassium. Hypertonicity of the body fluids is a favorable but not an essential condition for this response.

The authors wish to thank Dr. Samuel Harvey for the use of the animal room of the Department of Surgery, and for the assistance of its staff.

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EXPERIMENTAL HYPERTONICITY: ALTERATIONS IN THE DISTRIBUTION OF BODY WATER, AND THE CAUSE OF DEATH [1]

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In dogs deprived of food and water, water is lost out of proportion to the loss of salt. Concentrations of sodium and of chloride in serum therefore rise progressively for many days, until, just before death, concentrations as high as 186 m.Eq. of sodium and 133 m.Eq. of chloride per liter of serum have been found (1). It was not, however, clear whether death was due to the hypertonicity of the body fluids or to the concomitant dehydration. In certain of the present experiments, a comparable degree of hypertonicity has been produced acutely, without reducing the total body water. In others, hypertonic solutions were administered to animals already dehydrated. The reactions of the animals have been compared with those of the animals in which hypertonicity resulted from water loss alone.

MATERIALS AND METHODS

Twelve dogs in all were used. Chemical data relating to the fluid and salt exchanges of 8 of them are presented in the preceding paper (2). Two received 5 per cent saline intravenously, the rest intraperitoneally. Four of the animals had their ureters ligated just before the experiment. Dial anesthesia was used in 9 of the acute experiments, morphine in 1 acute experiment, and no anesthetic in the 2 chronic experiments.

After the anesthetic had taken effect, the animal was weighed, and a preliminary blood sample and electrocardiogram were obtained. Sodium chloride in 5 per cent solution was then injected intravenously or intraperitoneally. In some experiments, peritoneal fluid was removed after 30 or 40 minutes, in amounts approximating or somewhat exceeding the volume of saline injected. This fluid always contained salt in a concentration much lower than 5 per cent. Serial electrocardiograms were obtained from time to time. In some instances, further injections of saline were given. As far as possible, the animals were kept under continuous observation until death, while respiration, pulse, and other clinical data were noted periodically. Every attempt was made to be present at the exact moment of death and to obtain a terminal blood specimen and body weight. Sometimes the blood was obtained a little while before death.

Methods of chemical analysis, of measurement of plasma volume, and of estimating changes in intracellular, extracellular, and total water have been described in detail elsewhere (1, 4, 5). The symbols and formulae used have been summarized in the preceding paper (2), and will not be repeated here.

RESULTS

(a) *Changes in concentration of electrolytes and in distribution of body water*

The results of 4 typical experiments, 2 acute and 2 chronic, are presented in Tables I and II. In the 2 acute experiments (Experiments 27 and 28), 5 per cent sodium chloride was injected intraperitoneally with subsequent withdrawal of a comparable volume of peritoneal fluid, containing much less salt. The procedure resulted in a considerable addition of sodium chloride to the body without affecting much the total water content. The animals were then followed until death occurred, after 8 and 11 hours, respectively. In the 2 chronic experiments (Experiments 25B and 29), the animals received neither food nor water during the 8 days prior to the first intraperitoneal injection of 5 per cent saline. This solution was then injected in 50 cc. amounts, intraperitoneally, 2 or 3 times a day until death occurred, on the twelfth and on the fourteenth days, respectively. No peritoneal fluid was withdrawn. Dial anesthesia was used in the acute experiments but not in the chronic ones.

In the 2 acute experiments, serum concentration of sodium had increased by 40 or 50 per cent just prior to death (Table II). Chloride

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[2] National Research Council Fellow in the Medical Sciences, 1940–42.

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TABLE I

Exchanges of water, electrolytes, and nitrogen

Experiment	Time from start of experiment	Body weight†	Net intraperitoneal intake*			Urinary output			Balance	
			H_2O	Na	Cl	H_2O	Na	Cl	N	K
	days	*kgm.*	*cc.*	*m. eq.*	*m. eq.*	*cc.*	*m. eq.*	*m. eq.*	*grams*	*m. eq.*
27	0	7.85								
	0.3	7.83	125	364.2	369.3	0	0	0	− 0.10	− 2.1
28	0	6.64								
	0.5	6.24	−51	289.5	309.3	27	5.0	6.9	− 0.64	− 4.9
25B	0	8.52								
	8	7.12	−8	−1.1	−0.6	350	74.2	41.0	−12.20	−52.0
	10	6.82	111	122.3	124.0	120	85.4	76.0	− 2.67	− 9.6
	12	6.62	150	162.7	165.0	115	80.7	75.6	− 3.52	− 8.6
29	0	7.86								
	8	6.59	−8	−1.1	−0.6	230	48.0	23.8	−14.28	−46.7
	10	6.33	260	249.9	251.3	325	157.8	150.2	− 5.54	−23.7
	12	6.04	103	119.9	121.6	235	126.5	134.2	− 5.98	−17.3
	14	5.71	117		187.6	212		108.5	− 3.70	− 4.9

* Corrected for serum taken for analysis and saline solution of dye, T-1824, injected intravenously. In Dogs 27 and 28, peritoneal fluid was also withdrawn.

† Weights corrected for solids lost.

In both tables, time from start of experiment indicates end of period, at which time serum analyses were made and balances determined. Quantities are expressed per individual period rather than cumulatively. In designation of experiment, number refers to the individual dog; where a dog was used more than once, letter refers to successive experiments.

concentrations had risen to an even greater extent. In the 2 chronic experiments, the concentrations of sodium and of chloride, which had changed only slightly in the preliminary 8-day period of simple water deprivation, rose after repeated intraperitoneal injections of hypertonic saline to levels comparable to those found in the acute experiments. In the chronic experiments, unlike the acute ones, a large share of the injected sodium chloride was eliminated daily in the urine, so that the net positive balance of salt increased only gradually.

Extracellular fluid volume, whether calculated from the sodium or from the chloride balances, increased following hypertonic saline injections in all 4 experiments. In the 2 acute experiments, the increases were 70 and 40 per cent, respectively. In the 2 chronic experiments, there had been a decrease of the extracellular fluid volume during the 8 days of preliminary dehydration, and repeated injections of hypertonic saline were followed by a partial restoration of the extracellular fluid volume to normal.

Intracellular fluid volume declined in all 4 experiments (Table II). In the 2 acute experiments, the 2 independent methods which were used in the estimation of intracellular fluid change were in approximate agreement, the determination by means of the potassium balance giving somewhat lower results. Since, in both these acute experiments, there were only trivial changes in the total body water (Table II), the great expansion of the extracellular fluid was almost wholly at the expense of an equivalent reduction of the intracellular fluid. In the 2 chronic experiments, total water, intracellular fluid, and extracellular fluid had all decreased to approximately the same extent during the 8 days of preliminary simple dehydration. The subsequent injection of hypertonic saline resulted in still further depletion of the total water. Since extracellular fluid volume here too increased, there was a decrease of intracellular fluid exceeding that of the total water.

Measurements of plasma volume are presented in Table II. In the 2 acute experiments, only serum protein determinations are available. Concentration of protein dropped sharply; assuming no loss of circulating protein, this would mean that the plasma volume had expanded. In the 2 chronic experiments, total plasma volume was determined in two ways, from the distribution of Evans blue dye and independently from carbon monoxide distribution and relative cell volume. There was a moderate decrease of plasma volume during the

8-day period of dehydration. With the administration of intraperitoneal salt solution, plasma volume reexpanded. These changes closely parallel those of the extracellular fluid, and are opposed in direction to those of the intracellular fluid.

Hypertonic saline was given in a similar fashion to 8 other animals, in addition to the 4 which have just been described, with fatal results in 6. In Figure 1, the percentage changes of total water, of extracellular fluid, and of intracellular fluid have been compared with those in the 4 experiments which have just been described. The changes in plasma volume in Experiments 25B and 29 have been included for comparison. Extracellular fluid consistently increases, intracellular consistently decreases, and total water is little affected. Plasma volume changes parallel those in extracellular fluid. The extent of the distortion in distribution of body water is roughly proportional to the amount of extra salt in the body (abscissae).

(*b*) *Toxic effects on circulation and respiration, and the mode of death*

The peripheral pulse was slow and vigorous until the very end in both chronic and acute experiments (Experiments 27, 28, 25B, 29). In 3 of them, death occurred while the animals were actually under continuous observation. In all 3, death resulted from cessation of respiration 1 to 11 hours after the last injection of hypertonic saline, while the heart was still beating effectively. Shortly before the end, respirations began to be irregular and somewhat periodic. In the chronic unanesthetized dogs, there were muscular tremors, hyperactive reflexes, and some incoordination, but no true convulsions. The high plasma volumes just before death, the failure of the blood non-protein nitrogen concentration to rise, and the continued excretion of urine in the chronic experiments, all indicate that the circulation was functionally well-maintained until the end.

Serial electrocardiographic studies were made at intervals in these 4 experiments, and in 8 others as well. Some observations of pulse and of the manner of death were also made in these other 8 experiments. T wave changes, variable in extent and character, were usually encountered soon after injection. Fluctuation in the T wave pattern sometimes occurred during the course of the ex-

TABLE II

Analytical data, determinations of plasma volume, and calculation of changes in body fluid phases

Experiment	Time from start of experiment	Serum concentration					Blood		Plasma volume		ΔW_I*	ΔE_{Cl}†	ΔE_{Na}‡	ΔI_I§	ΔI_{II}‖
		Na	Cl	K	H_2O	Total protein	Hematocrit	Conc. NPN	CO method	Dye method					
	days	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	*grams per liter*	*grams per cent*	*per cent cells*	*mgm. per cent*	*cc.*	*cc.*	*liters*	*liters*	*liters*	*liters*	*liters*
27	0	140.2	104.3	4.33	936	6.51		15							
	0.3	193.3	170.5	8.03#	948	4.61		48			−0.02	+1.37	+1.36	−1.35	−0.90
28	0	136.0	102.3	4.80	941	5.92		30							
	0.5	212.8	185.8	5.32	949	4.64		41			−0.40	+0.73	+0.74	−1.13	−0.96
25B	0	143.4	109.9	5.19	935	5.86	41.3	25	523	548					
	8	144.9	117.1	4.59	932	6.83	47.4	26	368	409	−1.05	−0.45	−0.53	−0.60	−0.37
	10	155.8	127.8	4.59	942	6.08	42.8	20	362	426	−0.22	+0.21	+0.14	−0.43	−0.23
	12	179.6	133.3	4.31	933	5.44	31.0	19		454	−0.12	+0.50	+0.19	−0.62	−0.45
29	0	148.5	110.1	4.37	941	5.67	45.4	23	484	452					
	8	144.0	117.5	4.61	936	5.75	46.6	29	352	374	−0.95	−0.31	−0.28	−0.64	−0.28
	10	165.7	135.9	4.50	942	5.43	38.7	16	420	379	−0.18	+0.45	+0.36	−0.63	−0.50
	12	173.6	143.8	3.40	942	5.31	34.8	29	398	402	−0.22	−0.20	−0.13	−0.02	−0.20
	14	213.4	193.0	3.00	945	4.98	25.2	22		402	−0.25	−0.12		−0.13	−0.48

* ΔW_I = change in total water volume (Equations 1,2 (2)).
† ΔE_{Cl} = change in extracellular water volume (Equations 4,5 (2)).
‡ ΔE_{Na} = change in extracellular water volume (Equations 4,6 (2)).
§ ΔI_I = change in intracellular water volume (Equation 10 (2)).
‖ ΔI_{II} = change in intracellular water volume (Equation 11 (2)).
\# Blood drawn after respirations had stopped.

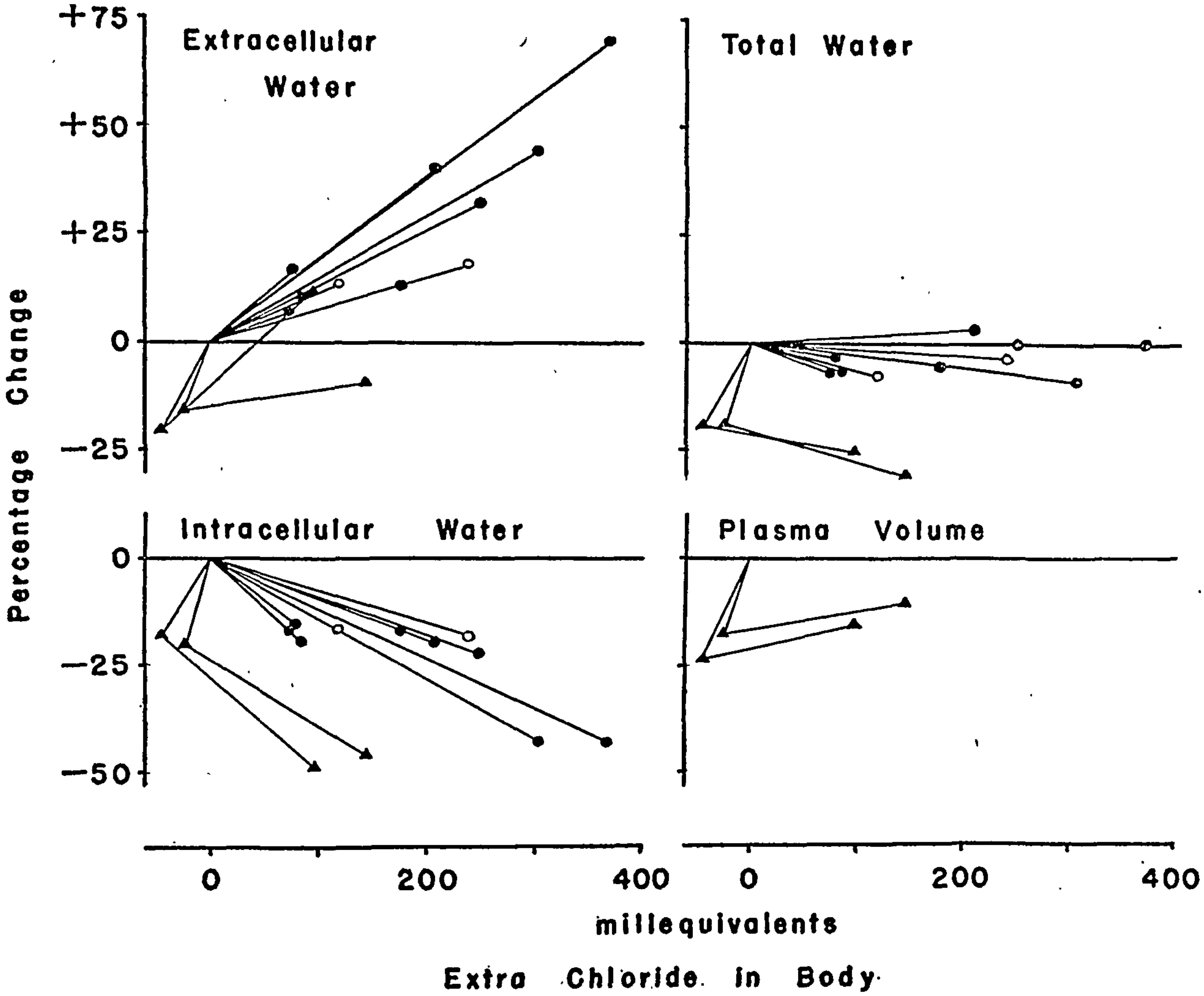

FIG. 1. COMPARISON OF THE PERCENTAGE CHANGES IN EXTRACELLULAR, INTRACELLULAR, AND TOTAL WATER VOLUMES, AND PLASMA VOLUMES, WITH THE AMOUNT OF EXTRA SALT IN THE BODY, IN 12 DOGS MADE HYPERTONIC BY THE INJECTION OF SODIUM CHLORIDE

Changes in volumes are plotted along the ordinates, while amounts of extra chloride are plotted along the abscissae. Solid black triangles represent the dogs deprived of water for a period before receiving the salt injections, open circles represent the 2 dogs that survived, and solid black circles represent the rest of the animals.

periments, even when the concentration of base or the distribution of water was not changing. Occasionally, a depression of the ST segment would appear immediately following the introduction of the hypertonic solution, but usually reverted to normal, even when no additional salt was given. Heart block developed terminally in one experiment, but this may well have been the result of an injury by a needle during a cardiac puncture. All in all, the electrocardiogram deviated very little from normal in any of the experiments, and just prior to death the complexes were usually essentially unchanged.

In Figure 2 are charted the concentrations of sodium, of chloride, and of potassium, at or just prior to death, in 10 experiments which resulted fatally. The maximal concentrations observed in the 2 dogs that eventually recovered after receiving large amounts of hypertonic saline are also included. In 2 instances (one associated with heart block, the other when blood was taken after respirations had stopped), there was a moderate increase in the serum potassium, while in the rest, its concentration was normal. Chloride and sodium were regularly markedly elevated, both roughly in proportion to the amount of extra salt in the body. There is no good evidence of any critical concentration, however, since death sometimes occurred at concentrations below those tolerated by the 2 dogs which survived.

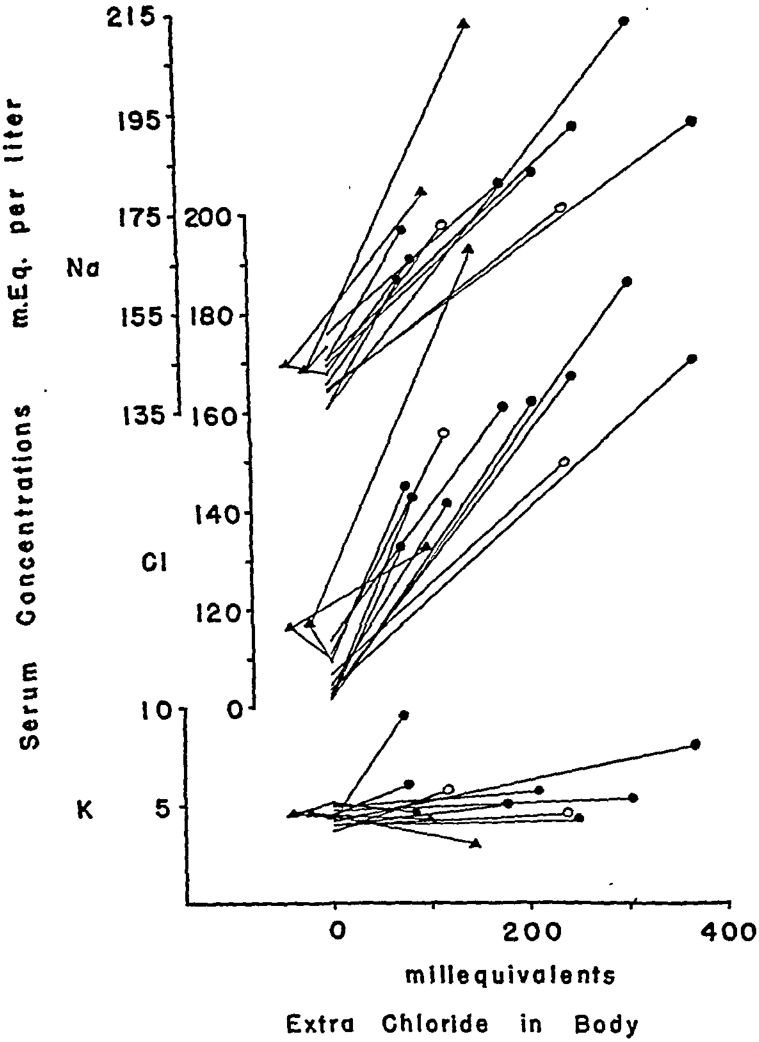

FIG. 2. COMPARISON OF THE CONCENTRATIONS IN SERUM OF NA, CL, AND K AND THE AMOUNT OF EXTRA SALT IN THE BODY, IN 12 DOGS MADE HYPERTONIC BY THE INJECTION OF SODIUM CHLORIDE

Concentrations in serum are plotted along the ordinates and amounts of extra chloride along the abscissa. Symbols are as in Figure 1.

DISCUSSION

Parenteral injection of hypertonic saline in sufficiently large amounts evidently may cause death. Whatever the ill effects produced by such hypertonic solutions, they clearly do not include impairment of the circulation. Death was apparently due to respiratory failure, the circulation remaining adequate until the end. This remarkably satisfactory state of the circulation in animals soon to die was associated with an expanded state of the extracellular fluid. Electrocardiographic changes were minimal, pulse was vigorous, plasma volume was high, and renal activity was well maintained. Clearly, there was no potassium poisoning (Figure 2). Even in animals previously dehydrated for 8 days, the circulation was in nowise depressed by repeated injections of hypertonic saline; indeed, the plasma volume actually increased slightly.

Hypertonicity in our experiments with saline injection was invariably associated with a reduction in intracellular fluid volume. No way could be devised to increase the concentration of extracellular salt without causing a simultaneous os-

motic shift from the intracellular to the extracellular phase. Injection of potassium salts would have been useless, since Eichelberger (3) has already shown that this procedure fails to expand the intracellular phase. Temporary hypertonicity of the body fluid, with minimal initial distortion of its partition between cells and interstitial fluid, was actually produced by injections of urea in concentrated solution (2). Urea crosses most cell membranes freely. With the amounts used, no deleterious effects developed. This is perhaps some indication that hypertonicity *per se* may be relatively unimportant. Also, the same amount of saline is required to kill an animal by intravenous injection as by peritoneal injection, yet the transient concentrations of sodium and of chloride during intravenous injection must have greatly exceeded those following intraperitoneal injection. This is another bit of evidence suggesting that the degree of contraction of intracellular fluid may be more important than the actual concentration of circulating electrolytes.

Certainly there must be some lower limit below which intracellular dehydration cannot be tolerated, since the complex metabolism of cells cannot continue in a dessicated state. Equally clearly, on the basis of our experiments (Figure 1) and of those reported by Elkinton and Taffel (1), cells can sometimes function until they have lost 40 to 50 per cent of their water. It is hard, consequently, to assess the degree to which a less severe contraction of intracellular fluid limits the organism. Reductions of intracellular volume of 16 to 20 per cent, found in 5 of the fatal cases, were also found in the 2 chronic dogs before receiving saline, and in the 2 acute dogs which survived. Reductions of this magnitude, therefore, seem hardly sufficient to be wholly responsible for death. Possibly in some cases, there was a synergistic action between the intracellular dehydration and the depression due to the anesthetic. It is also possible that dehydration is best tolerated when produced gradually. General loss of cell fluid may include dehydration of the cells of the central nervous system as well. If this were the case, dehydration of the cells of the respiratory centers might at least contribute to the respiratory arrest from which the animals die. Whether or not cellular dehydration in the brain and elsewhere is responsible for the fatal effect with hypertonic injections cannot, however, be decided without further experimentation. The dehydrated animals managed to survive for several days in spite of daily hypertonic saline injections.

Several mechanisms of adjustment were called into play to mitigate the deleterious effects of hypertonic injections. Perhaps the most important of these was the excretion of an abnormally concentrated urine, the chloride concentration at times exceeding 600 m.eq. per liter of urine. Although concentrations of this degree are unusual, the U/P ratio did not much exceed that commonly found in the dog, because of the simultaneous hypertonicity of the plasma. As a result, urine was excreted containing salt in concentration almost, but not quite, equaling that in the injected fluid. Urine volume was high enough so that total water loss proceeded about as fast as in the control animals receiving no injections. This urinary efficiency was only possible with an excellent circulation, which was in turn assured by the maintenance of large extracellular and plasma volumes.

A clinical state comparable to that of our animals might result from the consumption of sea water by man. Vomiting or diarrhea, if present, would of course have deleterious effects other than those studied here. If, however, any or all of the sea water were absorbed, our results are relevant. Assuming that the reactions of man are at least qualitatively similar to those of the dog, the sequence of events following the absorption of sea water may be outlined. At first, there would be an increase in the total water of the body, with a disproportionate increase in the extracellular fluid and a decrease in the intracellular fluid. Since the large extracellular fluid would favor an adequate renal flow, increased urinary excretion of water and salt would be expected. This would approximate in volume the amount of fluid absorbed, so that in the end dehydration would not be mitigated. The concentration of salt in the urine would presumably be less than that observed in our experiments, since the human kidney cannot concentrate urine quite as well as can that of the dog, and would certainly be less than its concentration in the ingested sea water. The final result would be an increase in the extracellular fluid, an equivalent decrease in the intracellular fluid, and an increase in the tonicity of all the

body fluids. It is not known whether death from respiratory failure has ever occurred in human beings who have carried this process far enough, but it is reasonable to predict its eventual development.

CONCLUSIONS

(*1*) Dogs may be killed by the introduction of a sufficient amount of sodium chloride into the body, provided the total body water does not change significantly.

(*2*) Introduction of sodium chloride, without change in body water, results in an increase in the volume of the extracellular fluid at the expense of a comparable decrease in the volume of the intracellular fluid. Both phases become hypertonic.

(*3*) The cardiovascular system is apparently unaffected by these developments. Electrocardiograms are little changed, plasma volume and renal function are well maintained.

(*4*) Death usually results from respiratory failure.

(*5*) It is suggested that intracellular dehydration, particularly affecting the cells of the respiratory centers, is the main but not the sole cause of death. There is no evidence for the existence of a critical lethal concentration of either sodium or chloride.

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PLASMA VOLUME OF DOGS IN DEHYDRATION, WITH AND WITHOUT SALT LOSS[1]

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Severe chronic water deprivation in the dog produces surprisingly little peripheral circulatory failure (1). This type of dehydration results in a contraction of extracellular fluid volume without any considerable sacrifice of extracellular salt. On the other hand, dehydration of somewhat comparable severity, in which salt as well as water is lost, may regularly be followed by acute circulatory collapse (2). From the published experiments, it is not clear whether this greater susceptibility of the circulation in the salt depleted animal is simply a quantitative matter, depending on a greater contraction of extracellular fluid and of plasma volumes, or whether it depends as well on the qualitative change in the composition of the body fluids. In the present experiments, reductions in body water were brought about, both with and without accompanying salt loss. The resulting changes in extracellular fluid volume, in plasma volume, and in the state of the circulation were then compared.

MATERIAL AND METHODS

The chemical methods used and the methods of calculating changes in the extracellular and intracellular distribution of water have been described elsewhere (1, 3). Plasma volume was determined by two independent methods. In the first, the volume of distribution of the blue dye, T-1824, 10 minutes after intravenous injection, was assumed equal to the plasma volume. In the second, the volume of distribution of a known volume of inhaled carbon monoxide, after 20 minutes, was assumed equal to the whole blood volume. Relative cell volume of anaerobically defibrinated whole blood was then determined in calibrated Daland hematocrit tubes (4). Plasma volume was calculated from the whole blood volume. Details of both methods are to be published elsewhere (5, 6).

Adult female dogs were used throughout. Intake and output of water, chloride, sodium, potassium, and nitrogen were measured. At suitable intervals, measurements of plasma volume were made, and changes in extra- and intracellular fluid volume calculated from the weight changes and chloride balances. In Experiments 11A, 11B, 12A, and 12B, the changes in extracellular fluid volume were also measured directly by the distribution of sulfocyanate and radioactive chloride and sodium, the results of which are reported in another paper (7). In one group of chronic experiments, 2 dogs were first subjected to 12 days of water deprivation and fasting (Experiments 11A and 12A). After rehydration and refeeding, the dogs were rested for a month. They were then subjected to an acute depletion of sodium chloride by withdrawal of peritoneal fluid following previous intraperitoneal injection of an isotonic glucose solution (Experiments 11B and 12B). This was followed by water deprivation and fasting for 3 days. Finally, the salt and water contents of the animals were restored to normal. These 4 experiments permitted a comparison of the effects of water deprivation with and without salt depletion in the same animals. In Experiment 22B, the procedure was varied somewhat. Here the animal, in addition to simple dehydration, was subjected to progressive depletion of salt by the peritoneal route. The results could be compared with those in control animals in whom there was no such additional salt loss (1).

Four acute experiments were performed. In 2 of them (Experiments 23A and 26A), 10 per cent urea in 5 per cent glucose solution was injected intraperitoneally, and an equivalent volume of peritoneal fluid was withdrawn some 4 hours later. This resulted not only in salt depletion, but in the absorption of a great deal of urea into the system as well. During the next few hours, this absorbed urea stimulated a marked diuresis, so that there gradually developed a large net depletion of water in excess of that of salt. The 2 other experiments (25A and 26B) served as controls, since no urea was injected with the glucose and hence no diuresis with consequent water depletion developed.

Criteria of circulatory status. Three classes of criteria were employed. (*a*) Direct measurements of plasma volume were made by two independent methods, and the changes in plasma volume in turn could be compared with changes in serum protein concentration and in relative cell volume[4] (6). Since a decline in plasma

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[2] Alexander Brown Coxe Fellow, 1941–42.
American College of Physicians Fellow, 1942–43.

[3] National Research Council Fellow in the Medical Sciences, 1940–42.

[4] Changes in relative cell volume reflect changes in the base concentration of the plasma, as well as changes

TABLE I

Exchange of water, electrolytes, and nitrogen

Procedure	Experiment	Time from start of experiment	Net intake					Urinary output				
			H_2O	Na	Cl	K	N	H_2O	Na	Cl	K	N
		days	*cc.*	*m. eq.*	*m. eq.*	*m. eq.*	*grams*	*cc.*	*m. eq.*	*m. eq.*	*m. eq.*	*grams*
H_2O depriv.*	11A	12	−15	− 3.7	0.5			880	75.8	62.2	130.0	35.0
Oral H_2O		14	1079#	− 2.8	− 1.1			567	3.1	7.4	4.0	4.5
Re-feeding		26	6224#		587.3#	339.3#	114.1#	2440		547.0	249.0	89.3
NaCl deplet.†‡	11B	3	164	−107.8	− 78.1	−2.8	−0.2	225	9.3	8.1	16.5	6.2
NaCl restor.§‖		4	1110	152.7	154.2			515	38.0	51.1	36.2	6.4
H_2O depriv.	12A	12	− 9	− 3.7	0.5			620	70.8	55.6	111.2	29.5
Oral H_2O		14	1013#	− 1.6	− 0.4			205	5.9	9.6	7.4	4.8
Re-feeding		26	6345#		554.8#	321.6#	113.6#	1510		506.0	229.0	53.0
NaCl deplet.‡	12B	3	41	−113.0	− 93.9	−4.3	−0.2	340	19.8	30.4	30.2	3.6
NaCl restor.‖		4	1405	152.8	154.2			700	36.6	37.0	33.2	9.1
NaCl deplet.‡	22B	0.2	120	− 95.6	− 77.9	−2.4	−0.13	30	0.5	0.5	2.1	0.90
H_2O depriv.		2	−16	− 1.8	− 1.1			460	3.2	4.0	12.0	7.36
H_2O depriv.		4	−36	− 4.7	− 3.3			95	4.2	1.3	29.3	5.97
NaCl deplet.‡		5	136	− 39.5	− 34.2	−0.9	−0.09	97	3.1	1.1	17.4	5.01
H_2O depriv.		8	−39	− 5.5	− 3.3			190	0.9	3.2	31.7	14.78
NaCl deplet.‡	23A	0.3	3	− 52.8	− 42.7	−1.2	19.02	340	0.5	2.2	3.3	7.01
H_2O deplet. (urea)		1	−28	− 4.0	− 2.7			400	1.0	4.1	20.5	12.10
NaCl deplet.‡	26A	0.2	65		− 35.2	−0.9	21.71	525		8.8	6.5	8.27
H_2O deplet. (urea)		1	−72		− 8.2			500		1.0	30.9	16.90
NaCl deplet.‡	25A	0.3	103	− 77.8	− 63.8	−2.8	−0.12	23	0.3	0.6	2.0	0.52
Oral H_2O		1	−42	− 4.8	− 3.1			245	0	1.2	4.5	1.46
NaCl deplet.‡	26B	0.3	− 9	−156.0	−145.0	−2.8	−0.17	265	4.1	9.3	4.1	0.72

* Abbreviation for *deprivation.*
† Abbreviation for *depletion.*
‡ By intraperitoneal injection of glucose solution and subsequent withdrawal of peritoneal fluid.
§ Abbreviation for *restoration.*
‖ By intravenous injection of saline solution.
\# Oral intake (food consisted of "Maro" meat mixture; for analysis see (3), EXPERIMENTAL PROCEDURE).

In both tables time from start of experiment indicates end of period, at which time serum analyses were made and balances determined. Quantities are expressed per individual period rather than cumulatively. In designation of experiment, number refers to the individual dog; where a dog was used more than once, letter refers to successive experiments.

volume regularly accompanies the development of peripheral circulatory failure, these measurements gave indirect information concerning the status of the circulation. (*b*) Observation of the femoral pulse and estimations of the rate of venous return from the extremities, as well as the general condition of the animal, gave direct evidence of the dynamic state of the circulation. These observations were qualitative rather than quantitative. Only great weakness or actual disappearance of the femoral pulse was considered indicative of a dynamically inadequate circulation. Likewise, only virtual inability to obtain blood in any quantity by puncture of the femoral or jugular veins was considered evidence of diminished return. (*c*) In experiments lasting a number of hours, the concentration of non-protein nitrogen in the blood serves as a rough measure of the average functional adequacy of the renal circulation during the period in question. It should be noted that the dynamic criteria serve only to distinguish severe circulatory impairment, and that moderate restriction of circulatory competence might escape detection.

in its volume. Allowance for this fact was made wherever necessary.

RESULTS

The experimental results are presented in Tables I and II. Simple deprivation of food and water (Experiments 11A and 12A) affected the distribution of water and of electrolytes in a manner which has previously been described (1). Volumes of both intracellular and extracellular phases diminished to about the same degree.

TABLE II

Analytical data, determinations of plasma volume, and calculation of changes in body fluid phases

Procedure	Experiment	Time from start of experiment	Body weight#	Serum concentration: Na	Serum concentration: Cl	Serum concentration: K	Serum concentration: H_2O	Serum concentration: Total protein	Blood: Hematocrit	Blood: Conc. NPN	Plasma volume: CO method	Plasma volume: Dye method	ΔW_I**	ΔE_{Cl}††	ΔE_{Na}‡‡	ΔI_I§§	ΔI_{II}‖‖
		days	*kgm.*	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	*grams per liter*	*grams per cent*	*per cent cells*	*mgm. per cent*	*cc.*	*cc.*	*liters*	*liters*	*liters*	*liters*	*l ers*
	11A	0	11.36	142.7	108.1	5.01	938	6.24	49.3	25		653					
H_2O depriv.*		12	8.86	149.6	112.8	4.26	932	6.82	45.2	27		517	−1.96	−0.63	−0.71	−1.33	−1.10
Oral H_2O		14	9.02	137.2	102.8	4.24	939	5.96		25			+0.27	+0.16	+0.25	+0.11	+0.27
Re-feeding		26	10.20	142.2	104.5	4.57	938	5.85	39.1	30		634		+0.31			+0.48
	11B	0	10.34	140.0	109.8	5.42	939	5.99	47.2	33		605					
NaCl deplet.†‡		3	9.34	137.3	98.5	5.38	929	7.01	59.4	43		436	−0.80	−0.51	−0.71	−0.29	−0.07
NaCl restor.§‖		4	9.70	143.2	105.3	3.82	946	5.05	37.4	21		543	+0.42	+0.79	+0.75	−0.37	−0.29
	12A	0	11.44	144.9	113.3	4.74	944	5.57	49.4	23		571					
H_2O depriv.		12	9.04	156.2	117.0	4.78	934	6.01	52.2	49		425	−1.84	−0.54	−0.71	−1.30	−1.09
Oral H_2O		14	9.50	131.9	99.2	4.39	946	4.96		29			+0.56	+0.37	+0.39	+0.19	+0.62
Re-feeding		26	10.50	137.5	106.4	4.16	944	5.46	45.9	26		580		+0.22			+0.51
	12B	0	11.06	137.5	111.2	4.80	942	6.07	46.2	25		597					
NaCl deplet.‡		3	9.80	130.5	85.4	5.12	920	7.32	64.4	153		349	−1.05	−0.63	−0.91	−0.42	−0.10
NaCl restor.‖		4	10.18	139.6	103.8	4.39	947	5.14	39.9	37		556	+0.45	+0.75	+0.76	−0.30	−0.40
	22B	0	14.60	144.0	107.6	4.28	962	5.08	57.1	31	682	570					
NaCl deplet.‡		0.2	14.47	133.5	96.2	3.75	941	6.17	61.3	27		544	−0.13	−0.38	−0.50	+0.25	+0.26
H_2O depriv.		2	13.36	143.9	105.0	4.76	937	6.42	56.8	30	518	585	−0.93	−0.32	−0.27	−0.61	−0.54
H_2O depriv.		4	12.51	149.3	107.0	4.78	936	6.72	59.9	39	558	462	−0.71	−0.11	−0.18	−0.60	−0.41
NaCl deplet.‡		5	12.39	144.7	97.8	4.48	931	7.09	54.6	56	568	510	−0.03	−0.08	−0.28	+0.05	+0.01
H_2O depriv.		8	11.40	142.4	102.3	5.30	934	6.57	51.8	54	549	415	−0.78	−0.16	±0	−0.62	−0.14
	23A	0	8.18	144.7	106.8	4.83	944	5.48	49.0	34	209	390					
NaCl deplet.‡		0.3	7.70	144.2	106.0	5.62	929	8.42	64.2	224	185	242	−0.48	−0.39	−0.39	−0.09	−0.06
H_2O deplet. (urea)		1	7.22	153.6	117.0	5.28	930	7.55	56.1	77	276	446	−0.43	−0.21	−0.13	−0.22	−0.29
	26A	0	9.30	140.6	109.9	5.41	954	4.59	35.0	32	617	599					
NaCl deplet.‡		0.2	8.78	139.2	110.4	6.27	941	5.78	44.4	202	488	415	−0.52	−0.40		−0.12	−0.11
H_2O deplet. (urea)		1	8.11	150.9	118.7	5.53	950	5.19	32.3	41	628	525	−0.62	−0.19		−0.43	−0.42
	25A	0	8.48	141.2	103.6	4.93	940	6.15	44.4	27	534	544					
NaCl deplet.‡		0.3	8.52	120.0	88.4	4.35	932	7.08	49.5	24	425	516	+0.04	−0.30	−0.29	+0.34	+0.49
Oral H_2O		1	8.31	125.6	89.2	4.58	940	6.03	41.8	26	440	540	−0.17	−0.04	−0.10	−0.13	−0.15
	26B	0	8.54	140.6	111.9	4.81	951	4.74	32.0	31	646	546					
NaCl deplet.‡		0.3	8.16	107.9	78.1	5.07	936	5.84	37.5	33	429	468	−0.38	−0.88	−0.86	+0.50	+0.82

*†‡§‖ See footnotes to Table I.
\# Weights corrected for solids lost.
** ΔW_I = change in total water volume (Equation 2 (3)); in Experiments 11A, 11B, 12A, 12B, ΔW is calculated as in Equation 1 (1).
†† ΔE_{Cl} = change in extracellular water volume (Equations 4,5 (3)).
‡‡ ΔE_{Na} = change in extracellular water volume (Equations 4,6 (3)).
§§ ΔI_I = change in intracellular water volume (Equation 10 (3)).
‖‖ ΔI_{II} = change in intracellular water volume (Equation 11 (3)).

Total ionic concentration in body fluids rose above normal. At the end of the period, the circulation was clinically almost unimpaired. Blood non-protein nitrogen concentration was unchanged in 11A and rose only slightly in 12A. However, the plasma volume, here measured by the dye method alone, was reduced by 21 per cent in one dog and by 26 per cent in the other. Serum protein concentration and relative cell volume changed relatively little. The stability of the latter may have resulted from a balance between the contraction of cells due to hypertonicity and the tendency of the relative cell volume to increase with decreasing plasma volume, or to an actual decrease in the total circulating red cell mass. All these changes in fluid distribution were reversed when fluid and food were restored.

Depletion of salt and water resulted in quite a different picture in the same 2 dogs (Experiments 11B, and 12B). Loss of water was mainly extracellular, and total ionic concentration of the body fluids fell. Intracellular fluid declined a little, since osmotic transfer of water into the cells did not quite compensate for that lost with protein breakdown. There was no "excess" loss of potassium ($b_{K'}$) over and above that to be expected from tissue breakdown alone (3). Both dogs were listless and weak, the pulses became weak and thready, blood was difficult to obtain by venepuncture, and blood non-protein nitrogen increased significantly. In both dogs, the changes in relative cell volume and in serum protein concentration exceeded those developing during the period of water depletion alone. The plasma volumes decreased 28 per cent and 42 per cent, respectively. In one dog, the decrease in plasma volume was only a little greater than that following water depletion alone, while in the other, it was much more marked. In both dogs, the evidences of circulatory inadequacy were all much more marked with salt depletion than without it. Injection of hypertonic salt solution and free drinking of water at the close of the experiment caused a re-expansion of the extracellular phase and of the plasma volume. During this period of restoration, there was a loss of potassium in excess of nitrogen, in spite of the net positive water balance. The significance of this has been discussed elsewhere (3).

The dog subjected to chronic water and food deprivation, together with progressive depletion of sodium chloride (Experiment 22B), died in the tenth day of the experiment. This was a shorter period of survival than that of control animals, deprived of food and water alone (In addition to the 2 control experiments described here, 4 have been reported previously (1)). Death occurred in spite of the fact that the total water loss (27 per cent) was less than that of the control animals. Water was lost from both phases of body water, but that from the extracellular fluid was proportionately greater. The dog appeared weak and listless, pulse was thready, venous blood flow was poor, and the blood non-protein nitrogen concentration was elevated. However, the final plasma volume was only moderately reduced, 19 per cent by one method and 26 per cent by the other. This reduction was no greater than that found without associated circulatory inadequacy in simple water deprivation (Experiments 11A, and 12A). The body fluids of this animal became chronically hypotonic, in contrast to the hypertonicity of the animals with simple water deprivation.

In 2 experiments (23A and 26A), in which urea administration was combined with salt depletion, there were 2 phases of reaction. In the first, salt depletion predominated, resulting in a transient hypotonicity with a moderate decrease in extracellular volume. Plasma volume also fell sharply. In the second phase, water loss exceeded salt loss, due to a diuresis induced by urea. Intracellular fluid volume decreased markedly and extracellular fluid volume continued to decline. As a result, the body fluids now became hypertonic. Despite the further dehydration, plasma volume returned to normal.

In the initial phase of 2 control experiments (25A and 26B), in which salt depletion was induced without subsequent water depletion, the diminution of extracellular fluid volume and the hypotonicity of body fluids were accompanied by a fall in plasma volume. Only 1 animal survived for a period corresponding to the second phase of Experiments 23A and 26A. In this phase, the body fluids remained hypotonic and the intracellular volume was larger than normal. The behavior of the plasma volume was indeterminate since, as measured by one technique, it rose, but, as measured by another, it remained depressed.

DISCUSSION

The view has been widely held that loss of sodium chloride is harmful mainly because it leads to a reduction in the volume of the extracellular fluid (8, 2). This is based on the fact that, under many circumstances, loss of extracellular water does tend to parallel that of extracellular salt. It is assumed that this reduction of extracellular volume leads in turn to a proportional contraction of plasma volume, with consequent impairment of normal circulatory dynamics. Our experiments indicate that this simple conception can be only partially correct. Were the reduced volume of the extracellular fluid wholly responsible for the adverse effects on the circulation, the manner in which the reduction was effected would be immaterial. Such is not, however, the case. For example, in Figure 1, the circulatory responses of *Dog 12 to simple* water deprivation and to loss of salt and water are compared. The decline in extracellular fluid volume was about the same with both procedures. Nevertheless, plasma volume was strikingly reduced and circulatory efficiency much impaired following salt withdrawal, effects which were absent *with simple* severe water loss. Here, obviously, changes in volume of extracellular fluid alone do not explain these differing circulatory reactions.

Clinical experience supports the importance to the circulation of composition as well as of vol-

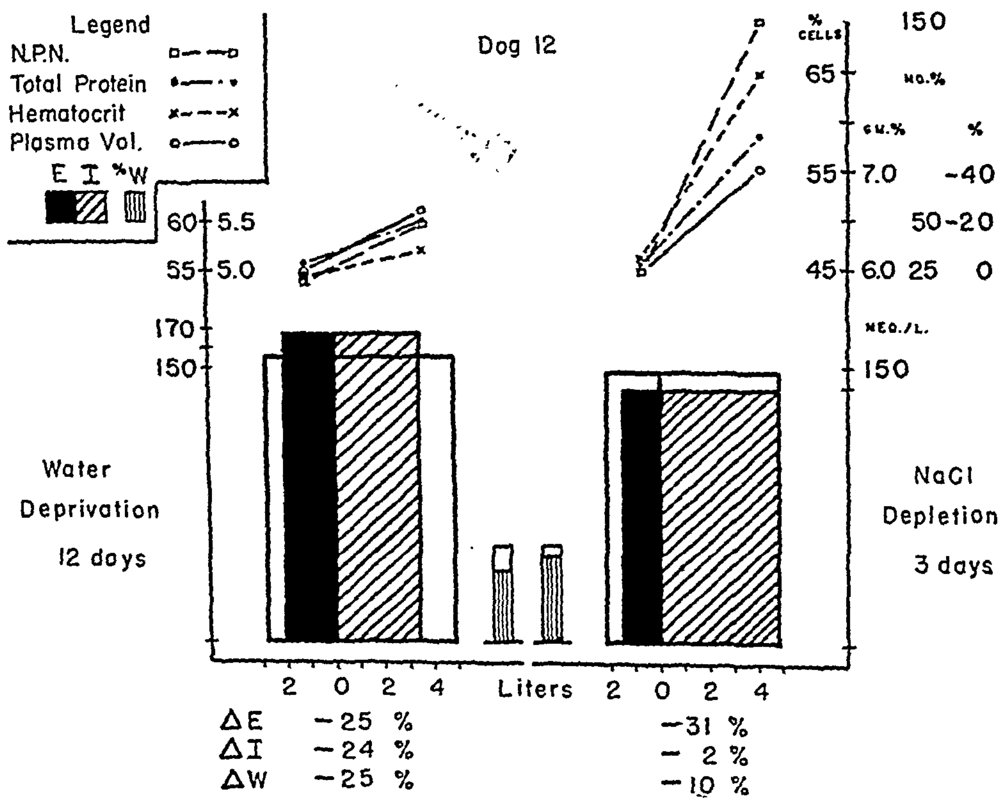

FIG. 1. COMPARISON OF THE EFFECTS ON THE PLASMA VOLUME AND ON THE CIRCULATION OF DEHYDRATION WITH SALT DEPLETION (RIGHT HAND FIGURE), AND WITHOUT SALT DEPLETION (LEFT HAND FIGURE)

The volumes of the extracellular (E) and intracellular (I) phases are plotted along the abscissae, while the total base concentrations are plotted along the ordinates. Areas, therefore, represent total amounts of electrolyte. The initial patterns are shown in outline, the final patterns in solid black and cross-hatching. Initial and final values for plasma volume, hematocrit, total protein, and non-protein nitrogen concentrations are plotted linearly. Percentage changes of different phases are given under the figure; Δ E, Δ I, and Δ W refer to changes in extracellular, in intracellular, and in total water volumes, respectively. Total water (W) is represented graphically by the small vertically-ruled colums between the two large diagrams.

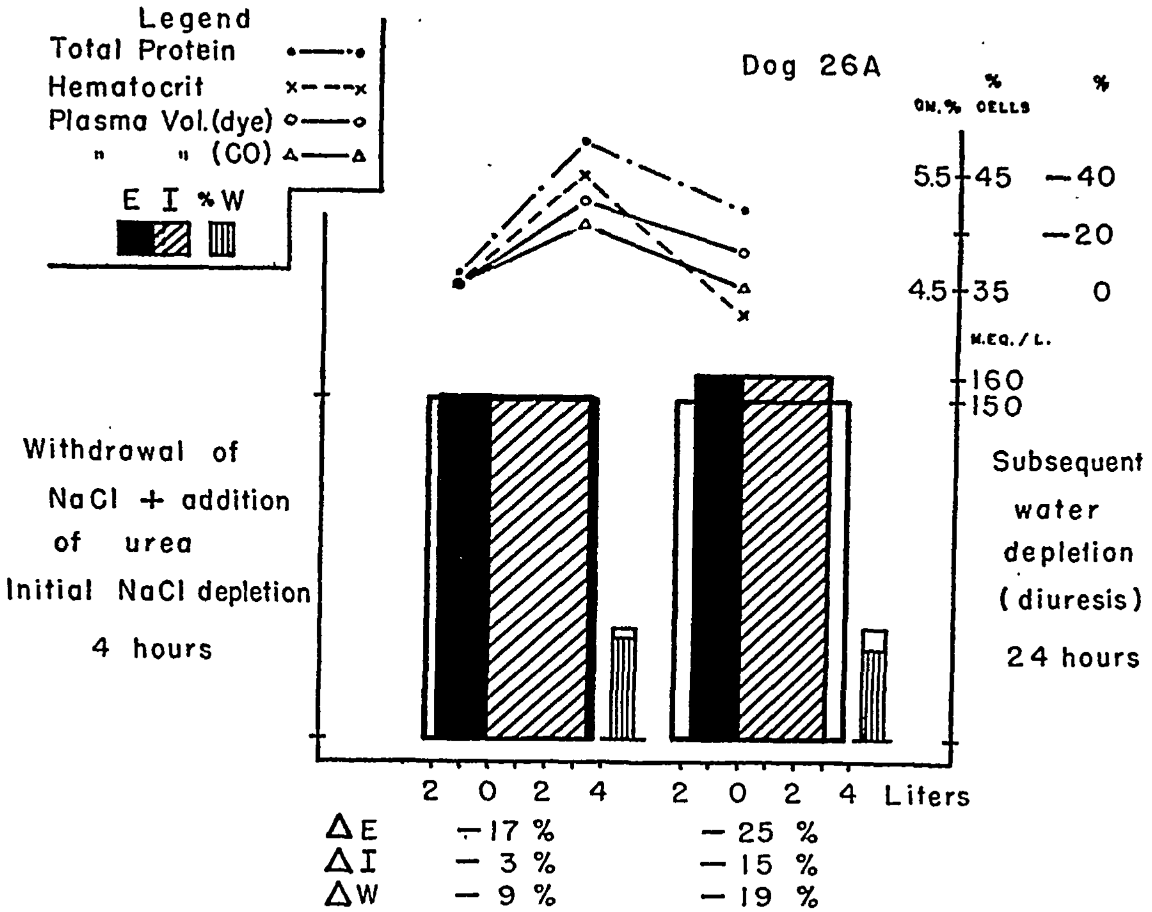

FIG. 2. THE EFFECT ON THE COMPOSITION AND DISTRIBUTION OF BODY FLUIDS, ON THE CIRCULATION, AND ON THE PLASMA VOLUME, OF INITIAL DEPLETION OF SALT AND SUBSEQUENT DEPLETION OF WATER (EXPERIMENT 26A)

Diagrams are similar to those in Figure 1, except that the body fluid pattern is represented at the end of the first period of 4 hours and again at 24 hours.

ume of the extracellular fluid (9). Parenteral administration of pure dilute glucose solutions to patients with shock associated with salt depletion tends to intensify rather than to alleviate the circulatory impairment. This is true in spite of the fact that such glucose solutions do effect some expansion of extracellular volume. The expansion is, however, effected at the price of a further dilution of extracellular electrolyte.

Extracellular fluid volume *per se* is evidently only one of the factors affecting plasma volume, and is not necessarily the most important one. Examples of dissociation between changes in volume of the extracellular fluid and changes in plasma volume may easily be found. Thus, the plasma volume of Dog 22B tended to increase on 2 occasions, although loss of extracellular fluid was progressive throughout the experiment. Plasma volume increased while the extracellular salt content of the body decreased in various other experiments, such as 22B, 23A, and 26A. Instead, contraction and subsequent reexpansion of plasma volume in the face of continued decline in the volume of the extracellular fluid is shown in Figure 2 (Experiment 26A). There is clearly a marked spontaneous tendency for the plasma volume to return to normal, even in the absence of sufficient water or salt.

Although peripheral circulatory failure was always associated with a sharp reduction in plasma volume, the converse of this proposition is not necessarily true. With simple dehydration, the plasma volume may be considerably reduced without rise in blood non-protein nitrogen or other distinct evidence of circulatory failure (Experiment 11A). It is probably mainly a question of the degree of contraction, since, even in this animal, a slightly greater contraction of plasma volume induced by combined water and salt depletion did result in some rise in the blood nonprotein

nitrogen (Experiment 11B). Granting that the contraction of plasma volume is the same, it is not clear that the circulation in the salt-depleted animal is functionally less adequate than that of the animal with simple water loss. There is certainly much to suggest that hypotonicity is harmful only in so far as it may favor a reduction in plasma volume. In Experiment 25A, for example, the circulation in the hypotonic animal seemed to be dynamically adequate so long as plasma volume was maintained. Conversely, there is little indication that increase in tonicity *per se* is responsible for improvement of plasma volume, since a rising plasma volume is as often accompanied by a fall in sodium of serum as by a rise (Table II).

CONCLUSIONS

(*1*) Changes in plasma volume parallel those in extracellular fluid volume only under certain limited conditions.

(*2*) Depletion of extracellular salt and water results in a more severe circulatory embarrassment than can be attributed to the contraction in extracellular fluid volume alone.

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GLOMERULONEPHRITIS. A SURVEY OF THE FUNCTIONAL ORGANIZATION OF THE KIDNEY IN VARIOUS STAGES OF DIFFUSE GLOMERULONEPHRITIS [1]

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Several studies of the functional changes which are encountered in chronic renal disease include observations on individuals with diffuse glomerulonephritis (1 to 3). However, the information on glomerulonephritis contained in such studies is meager. This fact, together with the diversity in the manifestations of the disease, precludes a description of its characteristics of renal function from the data available. The present study was designed to satisfy this deficiency in our information and has two specific ends in view. The first is to test the applicability of some of the more modern techniques of evaluating renal function to the situation obtaining in this condition. The second is to obtain a description of the characteristic pattern of functional activity of the kidney in glomerulonephritis. These ends have been achieved and it is now possible to formulate investigations of some of the more specific functional aspects of the disease by subsequent studies of a more limited scope.

Observations have been made on 22 patients with well-documented diffuse glomerulonephritis. The patients studied were selected so that the group contains a fair representation of the different stages of the disease, from a few weeks after its acute inception to shortly before its termination. The general and renal status of each patient were assayed by the usual clinical techniques, as well as by those which yield information on some of the discrete functions of the kidney. Among the latter functions are included the minimal renal plasma flow and blood flow, the glomerular filtration rate, and the maximal rate of tubular excretion of diodrast. These functions were studied in such a way that some of the important relationships between the discrete renal functions can be examined.

CASE MATERIAL

Patients were derived from several sources.[2] Each was observed for a sufficient period of time to insure a fairly secure diagnosis of diffuse glomerulonephritis before inclusion in the group. Some of the pertinent facts relative to the diagnosis, onset, and course of the disease in each patient are summarized in Table I. Information on the status of the patient just prior to the first experimental observation and the duration of the disease at this time is included (Columns 4 and 5). The type of the onset and the presenting symptoms and signs, as far as these could be determined, are noted, as well as the presence or absence of exacerbations (Column 6). The criterion for the latter diagnosis has been accepted as "an abrupt and marked increase in the degree of hematuria" (4).

Specific note is taken of infections in relation to the onset of the disease (Columns 7 and 8) and of the presence or absence of a nephrotic phase (Column 9) because of the bearing of these factors on the diagnosis. There is common agreement that the acute inception of diffuse glomerulonephritis and the exacerbation are typically preceded by an upper respiratory infection, generally associated with the presence of group A hemolytic streptococci. The latent period between the onset of the infection and that of the nephritis is usually 10 days to 3 weeks, while the latent period between the infection and the ex-

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[2] The authors are indebted to Dr. David Seegal of the 1st (Columbia) Research Service, Goldwater Memorial Hospital, New York City, for referring patients 4, 8, 11, 13, 15, 16, 17, 18, and 20 and permitting the use of the clinical records of these patients; to Dr. Duncan Clark and Dr. B. T. Tiernan of the Long Island College of Medicine for referring patients 1, 2, 12, and 22; to Dr. Abraham W. Victor of the Queens General Hospital, New York City, for referring patients 3, 6, 9, and 14; and to Dr. Shepard Shapiro of New York City for referring patients 10 and 19. Patients 5, 7, and 21 were drawn from the 3rd (NYU) Medical Service, Goldwater Memorial Hospital, New York City.

acerbation is 1 to 4 days (4, 5). Furthermore, a definite nephrotic syndrome is a common intercurrent manifestation of chronic diffuse glomerulonephritis, but except for true lipoid nephrosis is rarely found in other renal diseases (6). Generalized edema, serum albumin below 2.5 grams per 100 ml., and serum cholesterol above 400 mgm. per cent have been accepted in the present study as evidence of the presence of the nephrotic syndrome.

Information is also contained on the course of the disease (Column 10). The occurrence of infections during the period of observation (Column 11) is noted because of a possible influence on the progression of the disease (4, 5, 7).

The information available on each case (Table I) leaves little doubt of the validity of the primary diagnosis although the duration of the disease in each case is less certain.

EXPERIMENTAL PROCEDURE

The several renal functions measured were evaluated under standard conditions. The patients were maintained at bed rest and allowed no food or water from the time of the preceding evening meal until the completion of the experimental run. Experiments were begun between 8:00 and 9:00 A.M. and were from 2 to 2½ hours' duration. Studies were not performed if the patient's temperature was above 100° F. (rectal).

The routine procedure was as follows: Simultaneous mannitol and diodrast clearances were determined at constant plasma concentrations of each, during 3 consecutive periods of approximately 15 minutes' duration. The plasma diodrast level was then raised and after allowing 20 to 30 minutes for stabilization of the plasma diodrast concentration, diodrast Tm was determined in 3 additional 15-minute periods. Appropriate amounts of mannitol and diodrast to sustain the desired plasma concentration were administered throughout each experimental run by an infusion given at a rate of 2 ml. per minute. Mid-period bloods were obtained by venipuncture from the antecubital vein. Urine samples were obtained by catheter and followed, in all cases, by a bladder wash with sterile water.

The minimal renal plasma flow has been taken as equal to the diodrast clearance at low plasma levels (see below). Minimal renal blood flow has been calculated from this value and a hematocrit determination obtained at the beginning of the experiment (8, 9). Glomerular filtration rate has been taken as being equal to the mannitol clearance (8 to 11). The calculations involved in each of these measurements, together with that of diodrast Tm, followed the usual methods.

Tubular excretion of diodrast was examined in a limited number of experiments during a progressive increase of the plasma concentration of diodrast. A gradually rising plasma level was obtained as follows: An infusion containing a sufficient concentration of diodrast to sustain the plasma diodrast iodine at approximately 1 mgm. per cent was started at a rate of 2 ml. per minute. A more concentrated solution of diodrast was then added slowly by gravity to the initial infusion so that there was a progressive increase in the concentration of diodrast in the infusion fluid throughout the experimental observation. Thorough mixing of the infusion fluid was assured by the use of a stirring rod driven by a small electric motor. These arrangements may be adjusted to produce a steady increase in plasma diodrast of whatever rate is desired.

METHODS

1. Diodrast. Diodrast iodine in the plasma and urine was determined by a modification (12) of the Alpert method (13). Protein-free filtrates of plasma and urine were prepared using cadmium (14) and the filtrates treated with bromine in the usual fashion. The flasks containing the bromine-treated filtrates were chilled in an ice bath prior to the addition of KI and titration with thiosulfate. Chilling the solution just prior to the titration produces a sharp and relatively stable end-point for the titration.

Plasma contains a definite and measurable blank with this procedure. The blank is in the order of magnitude of 0.1 to 0.2 mgm. per cent diodrast iodine in the plasma of normal individuals but has been observed as high as 0.5 mgm. per cent in plasma from some individuals with excessive nitrogen retention. Because of the variability of the blank, it was determined routinely by an analysis of plasma, obtained prior to the administration of diodrast. A plasma filtrate was prepared in the usual manner and titrated subsequent to the addition of KIO_3. The urines of individuals with nitrogen retention may also contain a blank of considerable magnitude. This was estimated in a similar manner and expressed in mgms. of diodrast iodine excreted per unit time.

Recoveries of diodrast added to the plasma, when corrected for such a blank, were found to be quite constant but were only 94 per cent of the theoretical. These findings account for the fact that previous experience with the method (13) showed it to yield 100 per cent recovery at diodrast iodine values below 4 mgm. per cent, with progressively less at higher concentrations. The magnitude of the plasma blank is usually sufficient to balance the small loss of diodrast at the low plasma concentrations.

2. Mannitol and inulin. The method described by Smith, Finkelstein, and Smith (11) was used for the determination of mannitol except that the plasma and urine filtrates were oxidized at room temperature for 90 minutes (12) instead of for a shorter time in a boiling water bath. Protein-free filtrates of plasma were obtained using cadmium (14) except in the experiments where mannitol and inulin clearances were determined simultaneously. In these instances, both analyses were on a filtrate prepared with zinc (15). Inulin was determined by Harrison's (16) modification of the colorimetric method of Alving, Rubin, and Miller (17). Recoveries of known amounts of mannitol and inulin added to plasma and urine were excellent.

3. Plasma proteins. Total plasma protein and albumin

TABLE I

Clinical data concerning onset and course of diffuse glomerulonephritis

1	2	3	4	5	6	7	8	9	10	11
Case number	Sex	Age in years	Status of nephritis at first test	Duration of nephritis before first test	Onset of nephritis	Infection preceding onset of nephritis	Latent period	Nephrotic phase	Course of nephritis	Infections during observation
1	M	13	Healed	18 months	Acute: Smoky urine. Facial edema. Convulsion. BP: 170/125, fell to 110/60	Pharyngitis	3 weeks	No	Apparently healed 14 weeks after onset	None
2	M	25	Healed	4½ months	Acute: Smoky urine. Facial edema. Pain in back. BP: 160/100, fell to 115/70	Pharyngitis	3 weeks	No	Apparently healed some time after 2 months	None
3	M	14	Healing	5 weeks	Acute: Microscopic hematuria. Nocturia. Ankle edema. BP: 180/130, fell to 100/60	Pharyngitis	2 weeks	No	Apparently healed at or shortly after time of first test	None
4	F	13	Acute	3 months	Acute: Microscopic hematuria. Puffy eyes. Ankle edema. BP: 170/95, fell to 125/70	Pharyngitis (Group A hemolytic streptococcus proven)	2 weeks	No	Edema and hypertension disappeared but urine abnormalities persisted	Fever 99 to 100.6° until after 2nd test
5	M	22	Acute	3 weeks	Acute: Microscopic hematuria. Edema Dyspnea. BP: 160/106, fell to 115/80	Head cold Pharyngitis Otitis media	3 weeks 2 weeks ? days	No	Symptoms disappeared. Urine improving rapidly when last seen	None
6	M	12	Acute	6 weeks	Acute: Microscropic hematuria. Puffy eyes. BP: 170/120, fell to 140/70	Head cold	4 to 6 weeks	No	Apparently healed between second and third tests	None
7	M	44	Chronic	7 months or more	Acute (?Exacerbation): Microscopic hematuria. Facial edema. Ankle edema. BP: 170/100, fell to 140/85	Head cold	1 day	No	No change during observation. Retrograde pyelograms negative	None
8	M	14	Chronic	17 months or more 9 months	Insidious: Routine urine showed albumin. Facial edema. Ankle edema	?		Yes	Mild nephrotic syndrome throughout observation	Upper respiratory infection and catarrhal otitis media, 4 days after 1st test. Head colds at time of last two tests

TABLE I—*Continued*

1	2	3	4	5	6	7	8	9	10	11
Case number	Sex	Age in years	*Status of nephritis at first test*	*Duration of nephritis before first test*	Onset of nephritis	*Infection preceding onset of nephritis*	Latent period	Nephrotic phase	Course of nephritis	Infections during observation
9	F	41	Acute	2½ months	Acute: Gross hematuria. Generalized edema. BP: 170/90, fell to 120/80. (Previous urines and BP normal)	Pharyngitis	2 weeks	No	Steady clinical improvement throughout observation but urine did not clear entirely	Draining abdominal sinus one year, completely healed by third test
10	M	31	Chronic	8 years	Acute: Gross hematuria. Oliguria. Burning on urination	Pharyngitis	?	No	Recurrence of gross hematuria 4 years ago, microscopic hematuria ever since. I. V. and retrograde pyelograms negative	None
11	M	18	Chronic	6 to 7 years	Insidious: Ankle edema	?				
				13 months	Exacerbation: Microscopic hematuria. Facial edema. Leg edema. BP: 160/115, fell to 135/100	Pharyngitis	1 day	Yes	Nephrotic syndrome cleared up approximately 2 months before first test	Pharyngitis (No streptococcus demonstrated), 2 weeks before 1st test.
12	F	15	Chronic	10 months	Acute: Gross hematuria. Facial edema. BP: 140/104, fell to 115/80	Head cold	4 to 5 days	Yes		
					Exacerbation: Gross hematuria	Pharyngitis	1 day		Unobserved episode 7 months before last test suggestive of exacerbation. Asymptomatic at time of last test	Pharyngitis (not observed)
13	M	21	Chronic	1½ years	Insidious: Routine urine showed albumin (urine negative one year before)	?		Yes	Losing edema during observation	Chronic sinusitis and bronchitis throughout observation period
14	F	54	Chronic	6 months or more	Insidious: Anasarca	None noted		Yes	Nephrotic during observation, but edema responded in part to mercupurin	Cellulitis of leg, responded to sulfadiazine, 1 week before 1st test.
15	F	19	Chronic	4 years	Acute: Gross hematuria. Back pain. BP: ?	?		No	Four exacerbations in chronic glomerulonephritis	Chronic purulent sinusitis. 1 month before 5th test. Phlebitis leg, 1 month before 6th test. Pharyngitis twice (not observed)
				2 months	Exacerbation: Gross hematuria. Back pain. Facial edema. Ankle edema. BP: 170/100, fell to 120/60					

TABLE I—*Continued*

1	2	3	4	5	6	7	8	9	10	11
Case number	Sex	Age in years	Status of nephritis at first test	Duration of nephritis before first test	Onset of nephritis	Infection preceding onset of nephritis	Latent period	Nephrotic phase	Course of nephritis	Infections during observation
16	F	21	Chronic	10 months	Acute: Gross hematuria. Purpura. BP: 140/90	Pharyngitis	2 weeks	No	Marked progression of disease in 8 months between second and third test, no history of infection Died 7 weeks after last test. No autopsy	Grippe, temp.: 102° F. day before first test
17	M	35	Chronic	9 years or more	Insidious: Routine urine showed albumin	? "Grippe"	1 week	No	Hypertension first noted 4 years ago. Gradual progression of disease during observation. Died 7 months after last test Autopsy: Chronic glomerulonephritis	None
18	M	58	Chronic	2 months, probably much longer	Insidious: Anasarca	None noted		Yes	Died in uremia 3½ months after first test. Autopsy: Chronic glomerulonephritis	None
19	F	23	Chronic	6 years	Acute: Puffy face. Ankle edema. ? hematuria. BP: ?	Pharyngitis	?	Yes	Nephrotic phase cleared up approximately 6 months before first test	None
20	M	53	Chronic	6 years or more	Insidious: Intermittent ankle edema	None noted		No	Died in uremia 1 week after last test. No autopsy	None
21	M	47	Chronic	4½ years or more	Insidious: Ankle edema. Facial edema	None noted		Yes	Nephrotic phase cleared up 10 months before first test. Died in uremia 4 months after last test. Autopsy: Chronic glomerulonephritis and renal amyloidosis	None
22	M	24	Chronic	2 years or more	Insidious: Nocturia. Polyuria. Frequency.	None noted		No	Coma and nitrogen retention 6 months before first test. Retrograde pyelograms negative	None

were determined by the micro-Kjeldahl method (18) after precipitation by the Howe technique (19).

4. Quantitative estimations of the 12-hour urinary excretion of albumin and formed elements were performed by the method of Addis (20).

VALIDITY OF FUNCTIONAL MEASUREMENTS

The measurement of glomerular filtration rate. The plasma clearance of mannitol has been accepted, in these studies as a precise expression of the rate of glomerular filtration. This judgment was tentatively based upon those facts which lead one to suppose that the plasma clearance of inulin is such a measure in the normal (10) and diseased kidney (21) and the demonstration that inulin and mannitol clearances are identical in normal man and in women with preeclampsia and eclampsia (11). A similar situation appears to obtain in glomerulonephritis (Table II).

The comparison of the inulin and mannitol clearances was limited to 7 patients (mannitol clearances ranging from 8.0 to 104.0 ml. per minute) and were incidental to the routine observations. The two clearances were not completely identical in several of the patients. However, the differences are small and the filtrate fractions by the mannitol or inulin clearances are much the same. For the present purposes, then, the mannitol clearance may be considered to be an adequate measure of glomerular filtration in glomerulonephritis. It is accepted that for other purposes a more extensive comparison of the two clearances may be desirable.

These observations may be taken to indicate that the changes in the glomerular membrane in glomerulonephritis are not such as to preferentially interfere with the filtration of such a large molecule as inulin (10, 21) as compared to the filtration of a small molecule, such as mannitol. Similarly, the reabsorption of such a small molecule as mannitol is not facilitated by the changes in the tubular barrier.

The measurement of minimal renal plasma flow. The concepts underlying the use of the diodrast clearance for this measurement are well accepted. It may be stated arbitrarily that the diodrast clearance at low plasma concentrations, except in so far as the diodrast in erythrocytes contributes to that currently secreted, is a measure of the minimal renal plasma flow in any situation. However, its general usefulness in functional studies is largely derived from the fact that, in the normal kidney and under certain circumstances in the abnormal kidney, it also constitutes a close approximation of the actual renal plasma flow to the functional elements of the kidney (21). The measurement is still of some value in the absence of such a correlation, but the information in this circumstance is less generally useful.

The advanced stages of glomerulonephritis constitute a condition wherein the mechanism for the tubular transfer of diodrast is severely damaged, or the loss of renal tubular elements is so great, that the diodrast clearance at low plasma concentrations departs widely from the actual renal plasma flow. Calculations of the filtrate fraction in such a situation yield values which are higher than those which actually obtain at the glomeruli and which have little physiological significance. Such data are of little use in the examination of the hemodynamics of glomerular action.

TABLE II

Comparison of mannitol and inulin clearances in patients with diffuse glomerulonephritis

Patient number	Date	Mannitol clearance	Inulin clearance / Mannitol clearance	Filtrate fraction: Mannitol clearance / Diodrast clearance	Filtrate fraction: Inulin clearance / Diodrast clearance
		ml. per minute		*per cent*	*per cent*
9	October 28, 1942	104.0	1.07	17.2	18.5
9	September 21, 1942	94.0	1.08	17.1	18.5
11	November 1, 1942	88.0	1.04	20.0	21.2
8	August 21, 1942	57.4	1.00	12.0	12.0
13	September 2, 1942	53.0	0.99	11.9	11.9
15	August 31, 1942	38.9	0.97	8.5	8.3
19	October 23, 1932	17.8	1.06	17.6	18.6
18	August 24, 1942	8.0	1.06	11.5	11.9

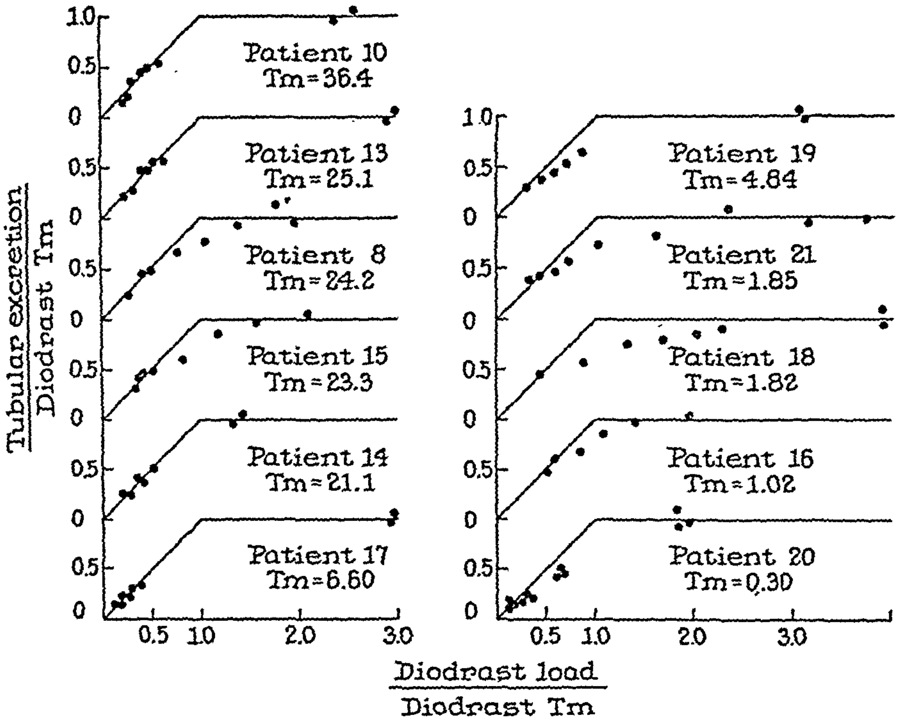

FIG. 1. THE TUBULAR EXCRETION OF DIODRAST IN RELATION TO THE LOAD OF DIODRAST DELIVERED TO THE TUBULES IN A SERIES OF PATIENTS WITH DIFFUSE GLOMERULONEPHRITIS

The normal relationship is shown by the solid lines. Tm values are uncorrected for surface area. The 6 experiments at the left of the figure indicate a straight line relationship between the load and tubular excretion of diodrast at diodrast load to diodrast Tm ratios below 0.5. The lack of this straight line relationship in the 5 experiments at the right of the figure indicates that true measurements of renal plasma flow could not be obtained in these 5 patients.

It was necessary, for these reasons, to establish the order of magnitude of the tubular impairment which is still compatible with an approximation of the renal plasma flow through a measurement of the diodrast clearance. This was accomplished by studying the tubular excretion of diodrast at different plasma loads in patients with varying amounts of residual renal tissue. The tubular excretion of diodrast in normal subjects is directly proportional to the load of diodrast presented to the tubules until the transport system for diodrast is completely saturated and no further increase in the rate of tubular excretion can occur, *i.e.*, diodrast Tm has been reached. This circumstance results from a situation which is such that, at the lower diodrast levels, the renal tubular excretion of diodrast is limited by the amount delivered to the renal tubules, while at the higher plasma levels the limitation is within the transfer mechanism itself (22). This relationship is indicated by the solid lines in the graphs of Figure 1 for the normal situation, wherein the transition between the two limitations is quite abrupt (22). The abrupt transition from one limitation to the other does not obtain in certain patients with diffuse glomerulonephritis, as evidenced by a falling off of the observed values from the normal curve as the diodrast load approaches diodrast Tm. This relationship was observed in all patients studied whose Tm values were below 5 mgm. per minute and in Patients 8 and 15 (Figure 1) where properly spaced data were obtained. Such a result may be partly a function of the mechanical distribution of blood to tubule tissue and partly the result of a limitation within the tubule cells whereby higher concentrations of diodrast in the extracellular fluid

TABLE III

Renal functions and related data in patients with diffuse glomerulonephritis

The discrete renal function values are corrected to a surface area of 1.73 sq. m.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
				Addis 12-Hour Urine Excretions																	
Patient number	Date	Status of glomerulonephritis at time of test	Blood pressure	Albumin *grams*	Erythrocytes *millions*	Casts *thousands*	Diodrast Tm *mgm. per minute* (Tm)	Renal plasma flow *ml. per minute* (PF)	Renal blood flow *ml. per minute* (BF)	Filtration rate *ml. per minute* (GF)	Filtration fraction *per cent*	PF/Tm ratio	GF/Tm ratio	Hematocrit, *per cent RBC*	Plasma proteins, *grams per 100 ml.*	Plasma albumin, *grams per 100 ml.*	Non-protein nitrogen, *mgm. per cent*	PSP excretion, 2 hours, *per cent*	Urea clearance, *per cent of average normal* ‡	Concentration test, max.	Dilution test, *minutes*
Average normal values[3] (9)							51.6	669	1115	131	19.6	13.0	2.54								
1σ							9.4	128	219	22.1	2.4	1.4	0.28								
1	August 7, 1942	Healed	110/60	0.04	3	16		744	1145	135	18.1			35	6.2	4.2	30	48		1.029	1.002
	August 17, 1942	Healed	110/70	0.04	1	15	57.6	775	1190	138	17.8	13.4	2.40	35	6.2	4.2	30				
2	July 31, 1942	Healed	115/70	0.06	1	0	56.4	782	1220	97.6	12.5	13.9	1.73	36	6.9	4.2	35	60		1.034	1.002
	October 2, 1942	Healed	120/70	0.05	1	0	56.0	993	1742	127	12.9	17.7	2.27	41	6.4	4.0	36				
	January 4, 1943	Healed	120/80	0.00	3	0	58.3	765	1296	109	14.3	13.1	1.87	41	6.6	4.2	24				
3	March 27, 1942	Healing	120/60	0.06	1	25	58.6	756	1260	114	15.0	12.9	1.95	40	6.8	4.8	22	63		1.025	1.002
	April 8, 1942	Healed	100/60	0.01	1	0	64.0	859	1387	130	15.1	13.4	2.03	38	6.8	5.0	31				
	April 20, 1942	Healed	115/70	0.01	2	0	59.8	712	1206	125	17.6	11.9	2.09	41	6.8	5.0	30				
	June 22, 1942	Healed	120/80	0.04	1	0	63.2	868	1335	150	17.9	13.7	2.38	35	6.9	4.3	31	47		1.027	1.004
	September 12, 1942	Healed	120/80	0.05	1	0	68.6	732	1240	126	17.2	10.7	1.84	40	6.8	4.8	30				
4	January 21, 1942	Acute	130/65	1.5	38	380	54.6	1244	1750	107	8.6	22.8	1.96	29	5.0	3.0	29	65	74	1.023	
	January 28, 1942	Acute	125/70	1.8	55	70	56.2	1230	1720	95.4	7.8	21.9	1.70	30	5.0	3.2	30				
	April 17, 1942	Chronic	124/84	2.6	22	0	60.4	813	1330	108	11.5	15.6	1.79	30	4.7	3.3	32	70	101		
	May 22, 1942	Chronic	130/80	0.7	9	52	56.6	921	1236	114	12.4	16.3	2.02	25	5.8	3.1	44				
	December 28, 1942	Chronic	120/80		10/HPF	0	64.2	936	1380	138	14.8	14.6	2.15	33	5.2	3.5	22				
5	June 29, 1942	Acute	115/80	0.33	14	370	46.9	722	1111	87.6	12.1	15.4	1.87	35	7.5	4.0	39			1.023	1.008
	August 12, 1942	Healing	120/80	0.06	18	0	52.4	626	920	101	16.6	11.9	1.93	32	6.5	4.3	44			1.028	1.001
6	March 16, 1942	Acute	150/70	0.12	221	70	46.8	452	741	61.3	13.5	9.6	1.31	39	6.6	4.8	28	67		1.021	1.004
	March 25, 1942	Healing	140/75	0.09	162	76	40.1	659	1030	84.9	12.9	16.4	2.12	36	6.7	4.8	25				
	May 13, 1942	Healing	145/80	0.03	30	41	47.1	718	1157	123	17.1	15.2	2.61	38	6.5	4.4	25			1.023	1.005
	June 24, 1942	Healed	140/70	0.02	2	30	48.6	838	1334	134	16.0	17.2	2.76	38	7.1	4.7	25				
	September 14, 1942	Healed	140/70	0.04	6	0	51.9	762	1270	122	16.0	14.7	2.35	40	7.4	4.6	39	39		1.030	1.003
7	January 2, 1942	Chronic	140/85	0.2	61	94		580	866	71.6	12.4			33				35		1.020	
	January 7, 1942	Chronic	160/95	0.1	55	19		622	929	82.0	13.1			33							
	January 16, 1942	Chronic	150/95	0.2	82		38.9	722	1050	84.3	12.0	18.6	2.17	33	6.4	4.0	28	51			
8	February 18, 1942	Chronic	125/70	3.8	11	370	38.4	564	778	69.6	12.4	14.7	1.81	28	4.1	2.0	29	60	56	1.020	1.006
	March 2, 1942	Chronic	115/70	3.2	7	408	33.8	661	816	64.5	9.8	19.6	1.91	28	4.2	2.0	32				
	April 27, 1942	Chronic	120/70	3.4	6	650	28.0	735	1020	67.6	9.2	26.2	2.41	28	4.0	1.9	29				
	May 11, 1942	Chronic	120/60	5.2	2	1390	26.5	751	1000	70.9	9.3	28.4	2.67	25	4.0	2.0	34	58			
	August 21, 1942	Chronic	122/72	3.2	3	5000	18.9	480	632	57.4	12.0	25.4	3.08	24	3.9	2.0	32		48	1.015	1.003
	January 11, 1943	Chronic	135/85	2.6	6	3500	19.5	498	664	72.8	14.6	25.5	3.73	26	3.9	2.1	33		52		
	May 8, 1943	Chronic	135/75	0.6	9	3500	18.1	333	483	55.4	16.6	18.4	3.06	31	4.8	2.9	33				

TABLE III—*Continued*

9	March 9, 1942	Acute	120/80	0.6	56	0	34.7	571	834	86.3	15.1	16.5	2.49	31	6.2	3.2	21	53		1.023	1.002
	March 23, 1942	Acute	120/80	0.17	40	106	34.3	563	840	81.1	14.4	16.4	2.36	33	6.9	3.9	22				
	April 27, 1942	Acute	125/80	0.10	40	33	41.3	596	932	84.9	14.2	14.4	2.06	36	7.0	4.1	22				
	September 21, 1942	Chronic	135/85	2.2	27	66	44.1	550	873	94.0	17.1	12.5	2.14	37	6.5	4.2	31				
	October 28, 1942	Chronic	120/75	0.15	21	0	53.4	603	972	104	17.2	11.3	1.95	35	6.5	4.5	29				
	January 15, 1943	Chronic	128/82	0.17	22	47		579	934	110	19.0			38	6.4	4.1	30				
	March 10, 1943	Chronic	130/85	0.20	16	0	44.7	601	910	107	17.8	13.4	2.39	34	6.6	4.3	30	60		1.034	1.002
10	April 24, 1942	Chronic	102/52	0.34	31	264	34.6	496	770	68.4	13.9	14.3	1.98	36	6.8	4.5	31	75		1.015	1.007
	May 1, 1942	Chronic	108/70	0.50	56	250	36.8	521	814	71.8	13.6	14.2	1.95	36	6.8	5.0	40			1.016	1.004
	September 11, 1942	Chronic	132/80	0.70	66	2200	37.9	453	755	74.5	16.5	12.0	1.97	40	6.5	4.6	38				
	January 26, 1943	Chronic	190/90	0.59	91	300	35.9	492	757	82.6	16.8	13.7	2.30	35	5.8	3.8	38				
	June 11, 1943	Chronic		0.70	61	600	34.7	690	1255	67.3	9.8	19.9	1.94	45	6.1	4.2	33				
11	February 4, 1942	Exacerbation	145/110	1.6	6	190	31.8	419	709	69.6	16.6	13.2	2.19	41	6.2	4.0	33	65	91	1.019	1.003
	February 9, 1942	Chronic	135/100	1.5	3	72	27.1	362	612	66.8	18.5	13.4	2.47	41	6.2	4.0	33				
	August 9, 1942	Chronic	160/115	3.9	1	1400	33.1	405	810	84.5	20.9	12.2	2.55	50	6.0	4.0	33				
	November 1, 1942	Chronic	160/105	2.6	3	3000	39.5	439	828	88.0	20.0	11.1	2.23	47	5.7	3.8	30				
	April 12, 1943	Chronic	150/100	0.8	2	1200	41.4	430	826	85.8	20.0	10.4	2.07	48	5.7	4.0	32				
12	July 29, 1942	Chronic	125/80	3.3	900	350	28.2	761	1103	85.0	11.2	27.0	3.01	31	4.1	2.3	27	54		1.029	1.001
	August 10, 1942	Chronic	115/80	5.5	980	72	21.3	646	872	68.3	10.6	30.6	3.21	27	4.4	2.3	30				
	June 22, 1943	Chronic	130/90	0.5	318	3000	2.3	*48	*66	18.1	*37.6	*20.6	7.77	27	4.8	2.8	32				
13	September 2, 1942	Chronic	144/100		126	31	21.9	447	732	53.0	11.9	19.1	2.27	39	4.7	2.8	44	70	55	1.020	
	September 18, 1942	Chronic	150/100	5.2	43	136	22.9	424	642	45.3	11.4	18.5	1.98	34	4.7	2.8	44				
14	July 8, 1942	Chronic	120/70	2.1	0	120	21.8	452	716	45.7	10.1	20.7	2.10	38	4.4	1.9	35	19		1.015	1.010
	August 17, 1942	Chronic	130/80	1.4	11	2100	20.2	374	526	34.1	9.1	18.5	1.69	29	4.1	1.9	41				
	November 25, 1942	Chronic	110/70	2.2	14	700	34.3	498	766	46.7	9.4	23.4	1.42	35	3.9	2.0	40				
15	May 8, 1942	Exacerbation	120/60	1.3	248	126	19.6	323	467	26.9	8.3	16.5	1.37	31	7.2	4.1	31	50	21	1.010	
	June 1, 1942	Chronic	130/70	0.8	266	0	26.7	325	458	32.3	10.0	12.2	1.21	29	7.3	4.0	41	45	26		
	July 1, 1942	Chronic	120/70	1.3	100	0	26.5	339	492	37.0	10.9	12.8	1.40	30	6.1	3.4	46	46	33		
	August 5, 1942	Chronic	115/70	0.7	49	0	30.2	306	419	40.5	13.2	10.1	1.34	27	6.2	3.9	45				
	September 23, 1942	Chronic	145/70	0.8	74	0	33.5	345	500	46.2	13.4	10.3	1.38	29	6.2	3.8	44		30		
	November 20, 1942	Chronic	130/70		14	120	27.6	231	345	39.3	17.0	8.4	1.42	33	6.0	3.8	40				
	May 15, 1943	Chronic	135/65		6/HPF	0	29.9	262	403	44.6	17.0	8.8	1.49	35	6.0	3.9	36				
16	January 19, 1942	Chronic	140/90	1.8	2660	580	17.4	268	412	52.1	20.5	14.7	2.85	35	5.0	2.9	34	43		1.024	1.004
	January 26, 1942	Chronic	120/90	2.5	934	520	15.1	290	446	46.5	16.6	19.2	3.08	35	5.5	3.5	35				
	October 5, 1942	Chronic	185/125	1.1	312	160	1.1	*28	*40	8.2	*29.5	*25.5	7.44	30	6.9	4.7	95	0			
17	March 6, 1942	Chronic	210/140	0.4	2	95	13.6	153	273	34.1	22.2	11.2	2.51	44	6.9	4.5	77	15	40	1.011	1.009
	March 18, 1942	Chronic	220/130	1.0	4	212	13.5	151	270	33.6	22.2	11.2	2.49	44	5.9	4.5	78				
	June 8, 1942	Chronic	240/160	3.8	4	0	7.9	94	145	21.7	23.0	11.9	2.73	35	6.9	4.5	78	25	51		
	July 10, 1942	Chronic	280/160		4	0	6.0	82	130	20.5	25.1	13.7	3.43	37	5.9	6.9	50				
18	June 17, 1942	Chronic	155/80	2.1	6	105	5.9	223	278	14.3	6.4	37.8	2.42	20	4.0	1.7	54	45		1.012	
	August 21, 1942	Chronic	176/84		8	0	1.8	*69	*91	8.0	*11.5	*39.2	4.52	24	4.4	1.8	67	0			
19	February 2, 1942	Chronic	135/90	0.4	46	100	5.7	*231	*329	22.2	*9.8	*40.2	3.87	30	5.6		61	15		1.010	
	February 6, 1942	Chronic	140/90	0.4	46	100				19.9							64				
	June 10, 1942	Chronic	140/90	3.3	56	220	5.8	*105	*146	19.7	*18.8	*19.2	3.60	28	6.1	3.5	61				
	October 23, 1942	Chronic	145/100	5.5	73	1140	5.1	*101	*133	17.8	*17.6	*19.9	3.50	24	5.7	3.6	49				
20	February 13, 1942	Chronic	230/140	3.6	239	0	1.9	*50	*62	5.7	*11.4	*26.5	2.88	19	6.4	3.3	80	0		1.011	
	February 23, 1942	Chronic	180/110	3.5	3176	0	1.6	*33	*40	6.5	*19.7	*20.6	4.07	19	6.3	3.3	180				
	April 1, 1942	Chronic	210/120		32	0	0.3	*8	*9	3.4	*42.5	*27.4	11.6	14	6.5	3.8	158				
21	April 21, 1942	Chronic	166/104	2.6	6	175	1.9	*42	*61	14.7	*35.4	*22.1	7.84	32	6.5	3.2	39	9		1.013	1.012
	May 18, 1942	Chronic	170/110	4.9	6	890	1.9	*41	*57	13.0	*31.6	*21.3	6.74	28	6.0	3.1	46				
22	October 21, 1942	Chronic	155/90	2.0	0	0	0.8	*15	*19	5.3	*35.3	*18.4	6.48	23	6.3	4.3	135	0			
	October 30, 1942	Chronic	150/90	2.2	15	90		*14	*19	4.4	*31.2			21	6.3	4.3					

are essential to saturate the transfer mechanism for diodrast (23), as in fact appears to be the case for phenol red in the normal subject.

It may be assumed for our present purposes that the maximal clearance of diodrast can only constitute a valid approximation of the renal plasma flow when determined at plasma concentrations below the level where the tubular excretion is directly proportional to the load of diodrast presented to the tubules. Also, it appears improbable from the data in Figure 1 that a valid plasma flow can be obtained with present chemical methods in these patients with Tm values much below 6.0 mgm. of diodrast iodine per minute. The lowest plasma levels of diodrast compatible with accurate chemical analysis may be expected to result in a tubular excretion of diodrast which, in these individuals, is not proportional to the plasma load. The apparent renal plasma flow of patients with a Tm value below 6.0 mgm. per minute has been recorded in the summary of results (Table III) but is marked by an asterisk, as are the derived relationships.

The estimation of filtrate fraction. This datum, being the dividend of the glomerular filtration rate and the renal plasma flow, is equal to the fraction of the plasma water which, reaching the glomeruli of the normal kidney, is filtered in the process of achieving pressure equilibrium across the glomerular membrane (8, 9, 24, 25). As such, the filtrate fraction, together with the absolute values of glomerular filtration rate and renal plasma flow, yields information on the hemodynamics of glomerular action. The value of the normal figure is 0.196 ($\sigma = \pm 0.024$). This is the net result of the operation of arterial pressure and mean resistance at the afferent and efferent arterioles, in so far as these define intracapillary pressure and those factors which determine the plasma oncotic pressure and the intracapsular pressure which oppose intracapillary pressure. Also concerned are those factors relating to the characteristics of the glomerular membrane and rate of plasma flow which, together with the pressure relationships, determine the degree to which pressure equilibrium is achieved in the glomeruli. Changes in the filtrate fraction from the normal value are, therefore, of some aid in the interpretation of the functional organization of glomerular action in any situation, providing the same significance can be attached to the data as in the normal subject. The latter appears to be probable in glomerulonephritis in those situations where the plasma clearance of diodrast has physiological significance (see above).

Diodrast Tm. Diodrast Tm may be accepted as a functional expression of the amount of tubular tissue (8, 9, 21, 25). Recently, it has been demonstrated that the magnitude of the diodrast Tm may be affected by non-renal factors. It seems likely, however, that the progressive, systematic changes in the diodrast Tm values which have been observed in this study are for the most part a reflection of the loss of renal substance or the alteration of renal tubular function as a result of the diffuse glomerulonephritis. This view is emphasized by the fact that significant changes in diodrast Tm in individual normal (9) or hypertensive subjects (26) are unusual over considerable periods of time.

RESULTS

The data obtained in each of the patients studied is summarized in Table III. The tabulation includes information on the renal plasma flow, renal blood flow, glomerular filtration rate and diodrast Tm (all corrected to a standard surface area of 1.73 sq. m.), the filtrate fraction, and the PF/Tm and GF/Tm ratios. The table also includes normal values for each of the functions studied [3] (9). The values for plasma flow, filtrate fraction, and the ratio PF/Tm which are marked by an asterisk are from studies where the evidence indicates that the diodrast clearance is no longer a close approximation of the renal plasma flow. The renal plasma flow and plasma flow to diodrast Tm ratio will be lower under these circumstances and the filtrate fraction higher than actually obtains. Other clinical data which bear on the status of the patient at the time of the clearance study are included in Table III. These observations were usually made within a day or two of the clearance study and in all cases were sufficiently close for them to be accepted as characteristic of that time.

The data indicate that a reduction in the amount

[3] The normal values for the filtrate fraction and the PF/Tm ratio were calculated from the renal plasma flow, glomerular filtration rate, and diodrast Tm figures, given in Table IV of the paper of Goldring, Chasis, Ranges, and Smith (9).

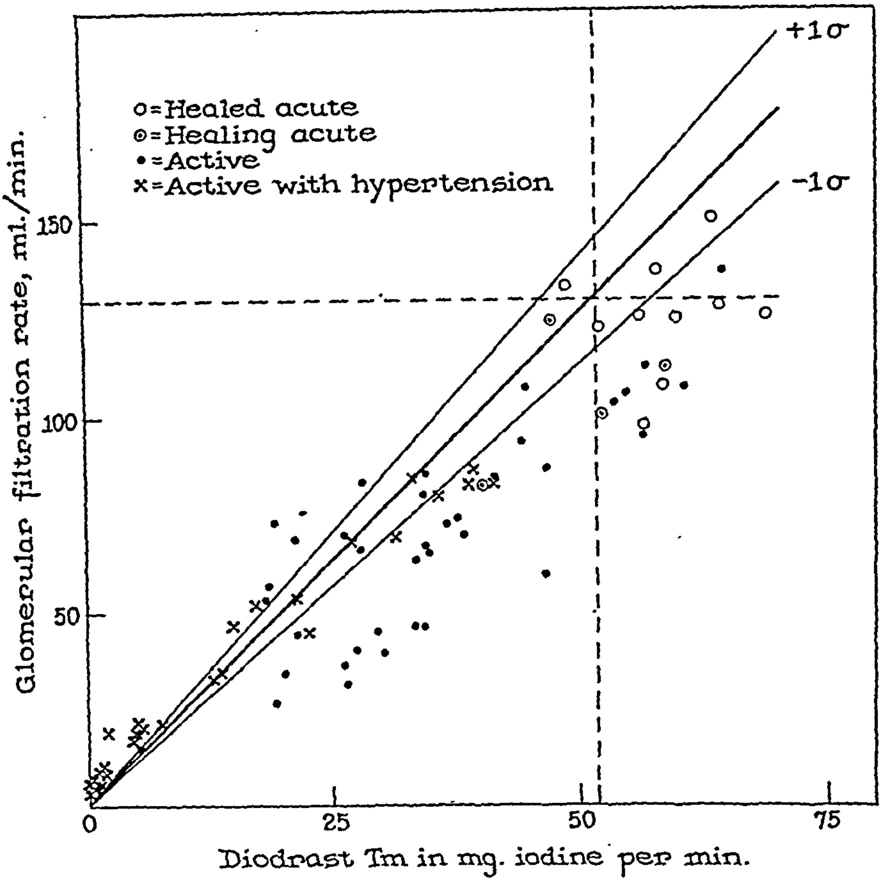

FIG. 2. GLOMERULAR FILTRATION RATE IN RELATION TO DIODRAST TM IN A SERIES OF PATIENTS WITH DIFFUSE GLOMERULONEPHRITIS [4]

The mean normal values for these functions are shown by the dotted lines; the normal relation between the 2 (GF/Tm = 2.54, $\sigma = \pm 0.28$), by the diagonal lines. Each datum in this and subsequent figures represents the average of 2 or more consecutive clearance periods.

of functional renal parenchyma, as measured by diodrast Tm, is usually accompanied by a roughly parallel decrease in glomerular filtration rate and in renal plasma flow; the former appears to be a much more sensitive indicator of the disturbance in renal function in this situation. Actually, a reduction in the renal plasma flow was not consistently noted until the diodrast Tm value was below 30 mgm. of iodine per minute. However, as renal damage progressed beyond this value for the diodrast Tm, there was a rapid decrease in the plasma flow to very low values. These relations are illustrated graphically in Figures 2 and 3.[4]

Figure 2 presents the relationship observed between glomerular filtration rate and diodrast Tm. The mean normal values for the variables are indicated by the horizontal and vertical lines; the normal relation between the two, by the solid diagonal line. Data obtained after the healing of an acute diffuse glomerulonephritis are indicated by open circles; those obtained during the healing or probable healing, by dotted circles; and the remainder (active), by solid circles, or by crosses when the diastolic blood pressure was in excess of 90 mm. of Hg. The data indicate that a reduction in glomerular filtration rate is generally accompanied by a decrease in the amount of functional tubular tissue, and also that patients with active diffuse glomerulonephritis characteristically have low GF/Tm ratios until the diodrast iodine

[4] Figures 2, 3, and 5 were patterned after those of Goldring, Chasis, Ranges, and Smith (26). See also footnote 3.

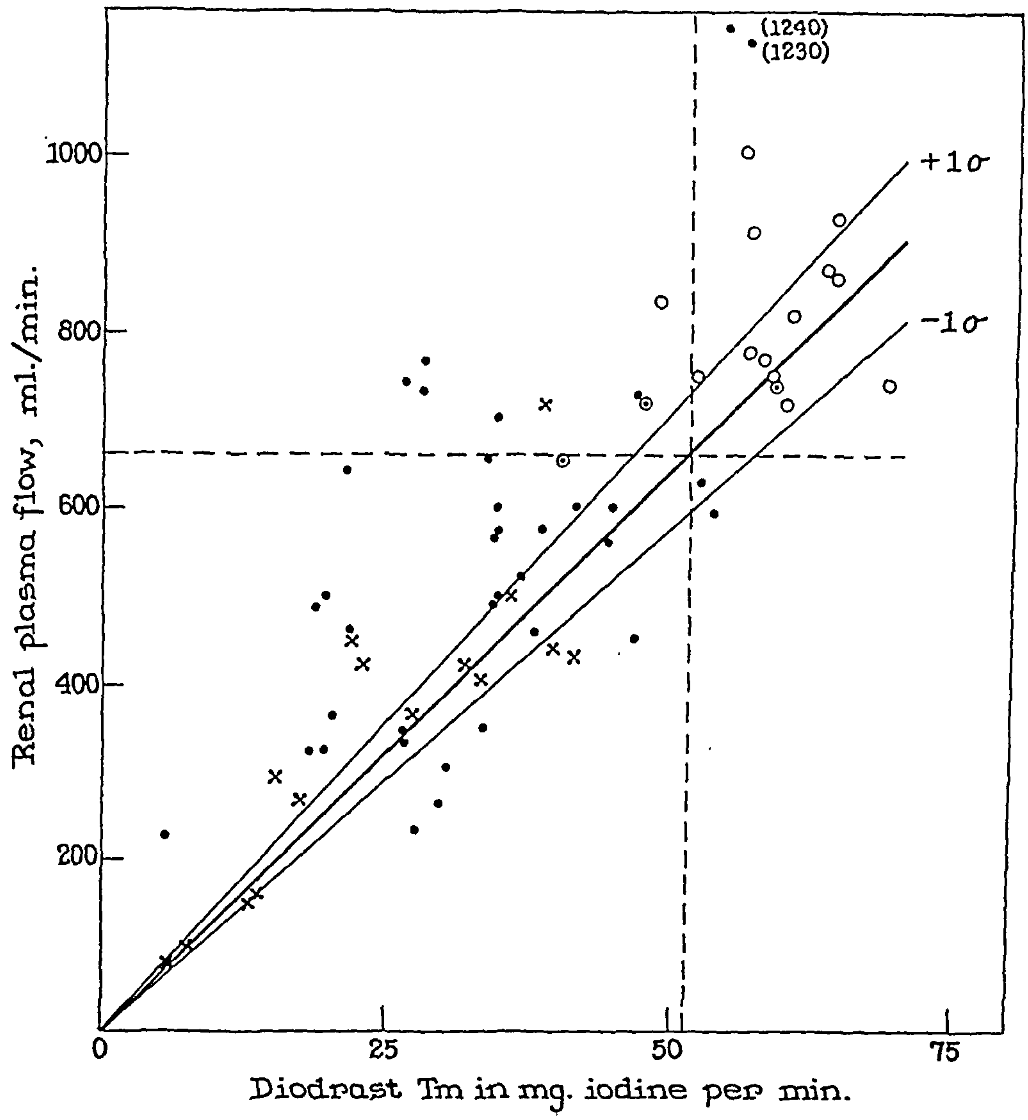

FIG. 3. RENAL PLASMA FLOW IN RELATION TO DIODRAST TM IN A SERIES OF PATIENTS WITH DIFFUSE GLOMERULONEPHRITIS [3, 4]

The mean normal values for these functions are shown by the dotted lines; the normal relation between the 2 (PF/Tm = 13.0, $\sigma = \pm 1.4$), by the diagonal lines. The status of the nephritis at the time of each observation is indicated as in Figure 2. Only valid renal plasma determinations are included.

Tm drops below 20 mgm. per minute. The progress of renal damage beyond this value involves a preferential loss of tubular tissue, or of some functional capacity of tubular tissue to do a specific type of work (*i.e.*, transfer diodrast), as compared to the ability of glomeruli to filter water and solute.

The relationship between renal plasma flow and diodrast Tm is summarized in Figure 3. This figure includes only those observations wherein the diodrast clearance is believed to be a close approximation of the renal plasma flow (see above). The general status of the nephritis at the time of the examination is indicated by different symbols, as in Figure 2. The mean normal values for the two variables are indicated by the horizontal and vertical lines; the normal relation between the two, by the solid diagonal line. The general reduction in renal plasma flow which accompanies the reduction in diodrast Tm is illustrated by the figure. However, it should be noted that high PF/Tm ratios are not uncommon in all stages of diffuse glomerulonephritis and also that when the status of the nephritis is complicated by the presence of hypertension, the observations do not depart from the usual relationship

in any systematic manner. It should be noted further that the scatter of the data is such that a close correlation between the two variables is not a characteristic of the pattern of renal function in glomerulonephritis.

A low filtrate fraction is one of the most consistent findings among these patients with diffuse glomerulonephritis, irrespective of the stage of the disease. This was still apparent in 1 (Patient 2) of the 2 patients studied only after healing, and in 15 of the 20 patients studied during the course of diffuse glomerulonephritis. The studies were made so late in the course of the disease in 2 of the 5 exceptions (Patients 21 and 22) that valid filtrate fraction values were not obtained. The figures recorded for these patients in Table III are higher than the true values.

The valid filtrate fractions obtained in this series of observations are plotted in relation to diodrast Tm in Figure 4. The mean normal filtrate fraction of 19.6 ($\sigma = \pm 2.4$) [3] per cent is shown by the solid horizontal lines. The general status of the nephritis at the time of the examination is indicated by different symbols as in Figure 2. The graphical summary emphasizes the frequency with which low filtrate fractions obtain in patients with active diffuse glomerulonephritis. Those with diastolic blood pressures above 90 mm. of mercury contributed almost all the normal and all the high filtrate fractions in the patients with diodrast iodine Tm values below 40 mgm. per minute. It should be noted, however, that hypertension is not inconsistent with a low filtrate fraction, as evidenced by the 3 observations with values of 0.12 or lower (Patients 7 and 13). The filtrate fraction is plotted against the PF/Tm ratio in Figure 5 and it may be seen that many of the observations fall outside and below the normal parameters, and also that the filtrate fractions of the patients with hypertension are distributed somewhat differently than the remainder.

The relation between the functional mass of tubule tissue (Diodrast Tm) and the diastolic blood pressure in the patients is shown in Figure 6. Diastolic hypertension does not appear to be a consistent finding, except during an acute episode, until diodrast iodine Tm falls below 20 mgm. per minute, although it is occasionally found

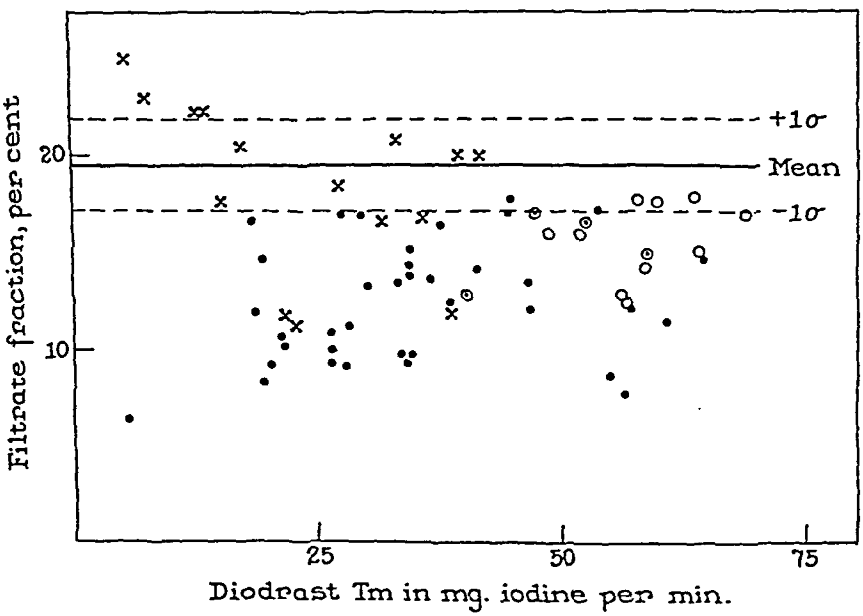

FIG. 4. FILTRATE FRACTION IN RELATION TO DIODRAST TM IN A SERIES OF PATIENTS WITH DIFFUSE GLOMERULONEPHRITIS [3]

The mean normal filtrate fraction (GF/PF = 19.6 per cent, $\sigma = \pm 2.4$ per cent) is shown by the horizontal lines. The status of the nephritis at the time of each observation is indicated as in Figure 2. Only filtrate fractions based on valid renal plasma flow determinations are included.

in nephritic subjects whose Tm values are considerably higher.

It has been shown that the renal vascular tree of normal individuals (24) and patients with essential hypertension (26) reacts to pyrogenic material in a manner which produces a marked increase in the renal blood flow. The renal hyperemia is not associated with a significant change in glomerular filtration rate, so that there is an associated fall in the filtrate fraction. Patients 9, 10, 12, and 15 were selected for a limited examination of this phenomenon in glomerulonephritis, and typhoid vaccine (24) was used as the pyrogenic agent. Three of the 4 patients showed a typical reaction, whereas there was a reduction of the filtrate fraction of Patient 12 which was a simple reflection of the fall in glomerular filtration rate. However, the injection of typhoid vaccine in this instance was followed by a severe general reaction and the observation was complicated by the incidental vascular phenomena.

It was anticipated that a study of the discrete renal functions and their interrelationships during the initiation and early stages of acute glomerulonephritis would permit an early differentiation of those patients who subsequently recovered completely, as compared to those who passed into a chronic phase. Such a differentiation has not been achieved in the data collected to date but it may be noted that information on the renal status of the patients during the first week or two following the inception of the disease is not available.

It is of some interest that, in the interval following the acute attack, there was no significant reduction of the absolute values of renal plasma flow, glomerular filtration rate, or diodrast Tm, in Patients 3 and 4, although the filtrate fractions and GF/Tm ratios did reveal abnormal relations. The nephritis of Patient 3 healed, the filtrate fraction rising to normal, but the nephritis of Patient 4 did not heal, although the filtrate fraction showed some progressive improvement. The remaining subjects with acute glomerulonephritis (Patients 5, 6, and 9) showed definite depressions of glomerular filtration rate or diodrast Tm, as well as of the filtrate fraction and GF/Tm ratio.

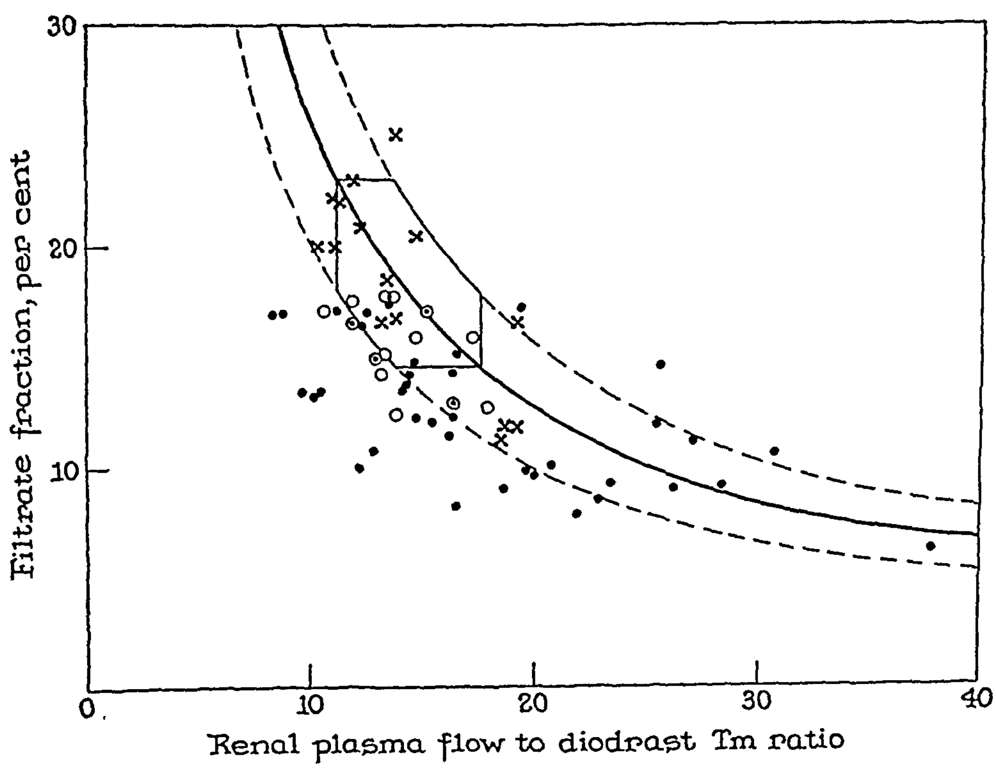

FIG. 5. FILTRATE FRACTION IN RELATION TO THE PLASMA FLOW TO DIODRAST TM RATIO IN A SERIES OF PATIENTS WITH DIFFUSE GLOMERULONEPHRITIS [3, 4]

The hexagon contains 95 per cent of normal basal data (26). The status of the nephritis at the time of each observation is indicated as in Figure 2. Only data based on valid renal plasma flow determinations are included.

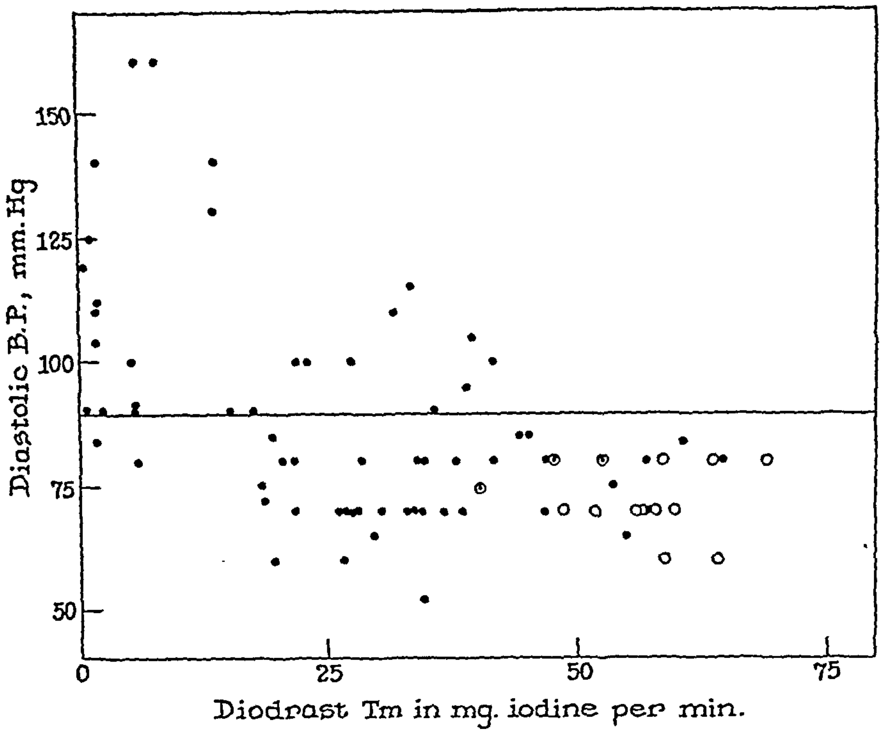

FIG. 6. DIASTOLIC BLOOD PRESSURE IN RELATION TO DIODRAST TM IN A SERIES OF PATIENTS WITH DIFFUSE GLOMERULONEPHRITIS

The status of the nephritis at the time of each observation is indicated as in Figure 2. The upper limit of normal diastolic blood pressure (89 mm. Hg) is indicated by the horizontal line.

The nephritis healed in Patient 6, while the process was definitely improving in Patients 5 and 9 when last studied. The discrete functions and the filtrate fraction and GF/Tm ratio in all 3 returned to normal or almost normal values and evidence of continuing kidney damage was derived largely from studies on the renal excretion of albumin and formed elements. Patient 2 still showed a low filtrate fraction 10½ months after the onset of an acute glomerulonephritis that healed according to the ordinary clinical criteria. There is, then, no apparent correlation in these patients between the degree of functional impairment at the time the patient was first seen and the eventual outcome of the acute glomerulonephritis.

Patients 11 and 15 were observed shortly following exacerbations in their chronic glomerulonephritis. The diodrast Tm gradually rose in Patient 11 to a low normal value during the 22 months following the acute episode. The glomerular filtration rate meanwhile rose slightly but did not attain a normal value. The exacerbation of Patient 15 produced a more severe depression of various functions studied and a greater distortion of their interrelationships. Some gradual improvement was noted in the rate of glomerular filtration and in the diodrast Tm, but these functions eventually stabilized at quite low values. The filtrate fraction rose from 8 per cent to a low normal value, but the GF/Tm ratio remained depressed. *An exacerbation also occurred in* Patient 12 during the period of observation. The effect of this episode on the progress of the disease could not be determined however, since 7 months elapsed between the time of the exacerbation and the *final renal function study.*

It is probable that more numerous studies in patients before, during, and after exacerbations will aid in determining the importance of such episodes in conditioning the overall rate of progression of the disease (4). The usefulness of measurements of these functional measurements in yielding information on the rate of the progression as well as on the amount of residual renal

tissue is brought out by the observations on Patients 8, 12, 16, 17, and 20. All of these patients were in the chronic phase of the disease. Patients 8, 12, 16, and 17 were observed serially over considerable periods of time and showed a steady decline in glomerular filtration rate and diodrast Tm. Patient 20, first seen in the terminal phase of the disease (Tm = 1.91), manifested a further decline in both glomerular filtration rate and diodrast Tm in the subsequent weeks, prior to death. The PSP test throughout the period of observation in this patient was zero. The declines in glomerular filtration rate in these patients would presumably have been reflected in similarly decreasing urea clearances had these been systematically observed.

In direct contrast to the patients who manifested a progressive impairment of renal function while under observation, Patient 10, studied 8 years after the initial diagnosis of chronic glomerulonephritis, showed no significant change in diodrast Tm and a slight and temporary but definite increase in glomerular filtration rate over a 9-month interval. The improvement of diodrast Tm in Patient 14 was more striking. Such improvements of the renal function of patients with long-standing chronic glomerulonephritis are difficult to understand and may represent improvement subsequent to undetected exacerbations.

DISCUSSION

A survey of 22 patients in various phases of diffuse glomerulonephritis indicates, as is to be expected, that the advance of the disease is associated with progressive depression of the renal plasma flow, the glomerular filtration rate, and the mass of functional tubular tissue as reflected by the diodrast Tm. The interrelations of the discrete functions also depart from the normal and yield additional information on the functional organization of the kidney in this disease.

Glomerular changes are perhaps the outstanding anatomical lesion of diffuse glomerulonephritis, early in the course of the disease. These changes commonly consist of a thickening of the capsule and basement membrane and an increased cellularity of the tuft. The arterioles of the glomeruli and the renal tubules are not extensively involved at this stage. The frequent finding of a reduced filtration rate, low filtrate fraction, and low GF/Tm ratio would appear to be a functional expression of such a morphological change. Progress in the disease is then accompanied by a progressive ablation of renal tubular as well as glomerular tissue, and all functions fall in a roughly parallel manner with the maintenance of an abnormally low filtrate fraction. The later stages of the disease appear to be accompanied by a greater acceleration in the loss of tubular rather than glomerular tissue or function. This circumstance is reflected in the high GF/Tm ratios and the relatively low values for the diodrast clearance. The latter stage of the disease is not amenable to simple analysis because of the difficulty in estimating renal plasma flow, as well as the frequent entrance of a complication in the form of a persistent hypertension. The latter may be expected to produce a disturbance in both systemic and glomerular hemodynamics.

The abnormally low filtrate fraction is a reflection of the glomerular changes which effect a greater barrier to the maintenance of glomerular filtration than to the passage of blood through the glomerular apparatus. Viewed in this light, it is not surprising that some of the patients manifest an actual renal hyperemia (Patient 4) whereas in others this is simply reflected in a low filtrate fraction and reciprocal changes in the GF/Tm and PF/Tm ratios. The low filtrate fraction may be attributed to changes in the character of the filtering bed which prevent the attainment of pressure equilibrium in each glomerulus, or it may be the result of those factors which determine the equilibrium pressure within the glomeruli. The increased renal plasma flow produced by typhoid vaccine in nephritic patients indicates that the glomerular arterioles are capable of normal reaction in this situation. It is difficult to define the relative importance of each of the various factors which are concerned in determining the change in the filtrate fraction. However, it would be in keeping with the usual pathological findings in diffuse glomerulonephritis to assign a major role to those factors which together determine the rate at which pressure equilibrium across the glomerular membrane is achieved, rather than to those factors which together determine the order of magnitude of the pressures across the membrane. It should be noted in Columns 15 and 17 of Table III that many of the nephritic patients

had low hematocrit and plasma albumin values. An analysis (not presented here) of the relation of these factors to renal plasma flow, glomerular filtration rate, and filtrate fraction suggests that changes among these are dependent upon the disease itself rather than upon any direct relationships between the two groups of factors.

The early impairment of glomerular filtration rate, the low filtrate fraction, and the low GF/Tm ratios of diffuse glomerulonephritis are opposed to the usual findings in essential hypertension (26, 27, 28). Examination of Table III and Figure 3 indicates that patients with diffuse glomerulonephritis, with or without hypertension, do not commonly have low PF/Tm ratios. The graphical plot of the data in Figure 5, however, suggests that the presence of a hypertension in patients with diffuse glomerulonephritis may change the dynamics of glomerular action in such a way that there is an increase in filtrate fraction, above values generally found among non-hypertensive nephritics. It may be noted in addition that hypertension is not necessarily related to a reduction of the amounts of functional tissue, as measured by diodrast Tm, except in that it become more common with progressive lowering of the latter function (Figure 6). However, diastolic hypertension is not found with any considerable regularity until the Tm has been reduced below 20 mgm. of diodrast iodine per minute.

The data upon which the above discussion is based are presented graphically in Figures 2 to 6 which contain all acceptable experiments, done in patients whose diagnoses of diffuse glomerulonephritis were reasonably certain. The status of renal disease in the various patients varied from healed acute glomerulonephritis to terminal uremia. In some patients, the disease was not stationary during the periods of recovery or improvement after acute episodes, while in others, progressive deterioration of renal function was observed. It seems likely, therefore, that the data presented give a fair cross-sectional view of the effects of diffuse glomerulonephritis on the functional organization of the kidneys.

The data reported in this paper contain certain more general implications which may be related to some of the clinical manifestations of diffuse glomerulonephritis. The discrete renal functions in normal subjects may be viewed as if they were occurring in a single nephron (25, 29). This opinion rests upon the demonstration that all nephrons in the dog (29) and probably in man (30) are continuously active, and that the reabsorptive capacities of the individual tubules are closely correlated with the ability of their attached glomeruli to filter (29). It may be expected, in consequence of this, that symptoms of renal injury may become manifest because of an absolute deficiency in the number of residual nephrons or because of a general distortion of the normal relationships of the component parts of some or all the nephrons. The data indicate that in glomerulonephritis all of these factors probably operate throughout the course of the disease and that its early stages are characterized by a loss of the normal balanced relationship between glomerulus and tubule in at least a major portion of the nephrons. An anatomical basis for this judgment exists in the presence of all possible combinations of glomerular and tubular injury, throughout the course of the disease (31).

It may be anticipated from these considerations that the distortion of the normal quantitative relationship between glomerular and tubular function has important consequences to those mechanisms which are important in determining the rate of excretion of water and electrolyte. This judgment presupposes that these factors are similar in man to those demonstrated for the dog, and that while diodrast Tm is probably not directly proportional to the ability of the tubules to reabsorb sodium and other electrolytes, it is likely that impairment of one tubular function will be accompanied by impairment of others. In nephritis, then, glomerular damage out of proportion to the impairment of tubular function should predispose to the retention of electrolyte and, incidentally, water, as is so common. The opposite combination, however, may contribute to the demineralization which is also seen frequently, but later in the course of the disease. It may be calculated, furthermore, that a small percentage of nephrons, continuously diuretic because of an impairment in electrolyte absorption (32), can result in a urine of low specific gravity. This could explain the relatively long persistence of impaired concentrating power, frequently noted after healed

acute diffuse glomerulonephritis. The final definition of these problems, however, awaits more extensive studies.

SUMMARY AND CONCLUSIONS

1. The glomerular filtration rate (GF), renal plasma flow (PF), and maximal rate of tubular excretion of diodrast (Tm) have been studied in a series of 22 patients in various phases of diffuse glomerulonephritis. There is a depression of all 3 functions as the disease advances, associated with marked distortions in their normal relationships. The glomerular filtration rate is the most sensitive indicator of renal change early in the course of the disease. This is reflected by a low filtrate fraction and a low GF/Tm ratio.

2. Acute glomerulonephritis and exacerbations in chronic glomerulonephritis may be associated with depression of glomerular filtration rate, diodrast Tm, filtrate fraction, and GF/Tm ratio. Any or all of these values may return toward normal as improvement or healing of the acute process occurs. There may be a transient hyperemia as well, indicated by a high PF/Tm ratio. No correlation has been apparent between changes in specific renal functions or their relationships in acute glomerulonephritis and the eventual outcome of the disease.

3. As chronic glomerulonephritis progresses, the tubular function undergoes relatively greater impairment than the glomerular filtration rate, indicated by high GF/Tm ratios and a relatively excessive lowering of the diodrast clearance.

4. The development of hypertension has not been specifically related to the residual kidney mass, although it is not infrequent when there has been more than a 40 per cent reduction in Tm.

5. Certain implications of the results have been discussed.

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OBSERVATIONS ON THE EPIDEMIOLOGY OF STREPTOCOCCAL PHARYNGITIS AND THE RELATION OF STREPTOCOCCAL CARRIERS TO THE OCCURRENCE OF OUTBREAKS[1]

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One of the most difficult problems confronting physicians interested in the control of infectious diseases is that of "post-infection" and "healthy" carriers. The epidemiology of diseases associated with streptococci is particularly baffling because of the frequent occurrence of these microorganisms in the throats of normal individuals. It is only since the development of serological methods for differentiating hemolytic streptococci into groups and types by Lancefield (1, 2) and Griffith (3) that accurate epidemiological studies of streptococcal diseases have become possible. Although several surveys of epidemic scarlet fever and streptococcal pharyngitis, in which the strains were identified by these serological methods, have been published, most of these reports cover mainly the period of the outbreak and do not include data on pre-epidemic carrier rates. It seemed worthwhile, therefore, to report observations covering a 6-year period on the incidence of carriers of various Goup A streptococci and their relation to the occurrence of outbreaks of streptococcal pharyngitis among groups of rheumatic children under close observation in a sanatorium.

LITERATURE

Since most observers did not have the opportunity of studying pre-epidemic carrier rates of Group A streptococci, no attempt will be made to review the literature completely.

Although the observations of Bloomfield and Felty were made before methods for the serological differentiation of streptococci were available, these authors described many of the epidemiological features characteristic of outbreaks of streptococcal pharyngitis (4, 5). No rise in the carrier rate occurred at the time that cases began to appear. The infecting dose was usually acquired as the result of prolonged close contact and the susceptibility of different subjects varied greatly. According to these observers, individuals did not become "contact" carriers unless infection had occurred. This point of view has been reaffirmed in a recent article by Bloomfield and Rantz (6).

Dudley studied the incidence of scarlet fever and diphtheria in a boys' school (7). No cases of scarlet fever or diphtheria developed among the 100 day pupils, whereas both diseases were prevalent among the 300 boarding pupils. This difference was explained by Dudley in the following way: the natural defenses of the body are able to destroy small numbers of the infecting agent. When contact is prolonged, however, the velocity of destruction may be less than the rate at which the pathogenic bacteria are acquired, so that eventually the minimal infecting dose is reached.

In studies of epidemics of scarlet fever occurring in Rumania, Schwentker was able to obtain data on pre-epidemic carrier rates (8). He found that a definite rise in the carrier rate of the epidemic type preceded outbreaks. In the opinion of this observer, a marked increase in the number of carriers in a community carrying a single type of Group A streptococci may be used as an indication that an epidemic is imminent.

MATERIAL

The type of institution and the bacteriological procedures have been previously described (9). The relationship of the outbreaks of streptococcal upper respiratory infections to rheumatic relapses has also been reported (9, 10). From an epidemiological point of view, the following procedures and data are important. With only occasional exceptions, no new children were admitted during the winter months, *i.e.*, from November or December until the end of May or the middle of June. Each year the same 108 children, ranging in age from 7 to 15 years, remained in the institution for 6 months or longer and thus constituted essentially a "closed" colony.

The children slept in 5 wards of 18 to 24 beds each and

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ate in one large dining room. They attended school in 2 rooms in the same building. The 66 girls were divided into 3 and the 42 boys into 2 recreational groups. Children were usually admitted in groups of 8 and were isolated for 48 hours. During this time 2 throat cultures were taken to determine the presence of Group A streptococci. Thereafter, weekly throat cultures were taken routinely on every child throughout the year. When Group A streptococci were first isolated from a child, who had no complaints or clinical symptoms of an upper respiratory infection, the following tests were used to determine the presence of subclinical infection: leukocyte counts, erythrocyte sedimentation rates, and determinations of antistreptolysin O titers. If the laboratory findings remained unchanged, the child was considered to be a "healthy" carrier. In the case of known Group A streptococcus carriers, when a different colony form was observed on a routine throat culture plate, suggesting a possible change in type, similar laboratory procedures were employed to rule out the possibility of subclinical infection. Throat cultures were taken on 2 successive days on all children who showed symptoms of any kind. Nasal cultures were taken only when clinical evidence of nasal infection was present.

Attempts were made to group and type all hemolytic streptococci isolated. The details of the methods used have been published elsewhere (11). Streptococci isolated from known carriers were checked serologically once a month and more often when clinical or laboratory evidence suggested a possible change of type.

Classification of carriers

For the purposes of this study, carriers of Group A streptococci are divided into 3 categories: "post-infection," "contact," and "healthy." "Post-infection" carrier refers to children who develop streptococcal pharyngitis while under observation and then become carriers. "Contact" is used to designate individuals in the institution who, without developing clinical or laboratory evidence of infection, acquire streptococci either from other children with streptococcal upper respiratory infections or from known carriers. The term "healthy" carrier is restricted to children harboring streptococci on admission or shortly thereafter since it was not known whether they were "post-infection" or "contact" carriers.

During each year, the weekly incidence of all streptococcus carriers in the group was determined. Carriers were classified as temporary if they harbored hemolytic streptococci less than 2 months, and as chronic if for longer periods. Chronic carriers were further classified as intermittent or consistent. Observations based on 1 or 2 cultures gave an inaccurate picture of the number of carriers in the community. Not only may streptococci from intermittent carriers be missed, but even fairly consistent carriers occasionally fail to show these microorganisms. Weekly throat cultures obtained from chronic carriers may be negative for as long as 12 weeks and then become positive. Furthermore, irrespective of whether the carrier had clinical signs of a "common cold," marked variations in the number of streptococci isolated at different times from the same child were observed. In our opinion, therefore, the assumption that a particular carrier is not a possible source of danger, because 2 or 3 cultures yield only a few colonies of streptococci, is unwarranted.

EPIDEMIOLOGICAL FINDINGS

During the winters of 1937–38 and 1942–43, only a small number of cases of streptococcal pharyngitis occurred. The spread of infection during these 2 years differed from that observed during the 4 intervening years when the incidence of streptococcal pharyngitis was greater. The 2 years 1937–38 and 1942–43 are therefore grouped together and will be described later. The 4 winter seasons from 1938 to 1942, when the incidence of streptococcal upper respiratory infections was fairly high, are discussed first.

A. *Major outbreaks of streptococcal pharyngitis due to types not previously in the community*

1. *Outbreak of streptococcal pharyngitis due to type 4, February to June 1939*

Group A streptococcus carriers in the community on February 1, 1939. The 108 patients included 66 "new" (*i.e.*, those admitted during the summer and fall months) and 42 "old" children (*i.e.*, those who resided in the institution during the previous winter). Twenty-one of the 66 "new" children were "healthy" carriers of Group A streptococci. The following types were represented: types 5, 12, 17, 18, 19, 23, 28, 29, and 32, and 11 strains of undetermined types. In some instances, when a child became a carrier several weeks after admission, it was impossible to tell whether he was an intermittent "healthy" or a "contact" carrier who had acquired the strain from another child carrying the same type or from some unknown source, such as visitors or staff.

The carrier rate fell during the course of the winter so that by February 1, only 8 of the 21 "new" children who had been "healthy" carriers of Group A streptococci still harbored these microorganisms: 4 carried, respectively, types 5, 17, 19, 29, and the others, strains of undetermined types. Three of the 42 "old" children were chronic "healthy" carriers: one girl carried type 18 for more than 2 years; 2 other children carried streptococci of undertermined types for 21 and 22 months, respectively.

Thus, in February 1939, 11 of the 108 children, or 10 per cent, were carriers of Group A streptococci.

Outbreak of pharyngitis due to type 4 streptococcus, February 4 to May 15, 1939. On February 4, a girl developed acute pharyngitis due to type 4 streptococcus, a type not previously present in the community. It was thought possible that the child contracted the infection from her parents who had visited her 2 days before symptoms appeared. Three other girls developed pharyngitis due to type 4 streptococcus during February. The infection spread to the boys during March and reached its peak with 17 patients: 8 girls and 9 boys. In April and May, the incidence declined to 6 and 5 cases, respectively, and was confined mainly to the girls (9 girls and 2 boys). Thus, during 4 months, a total of 32 cases occurred among the 108 children.

The monthly incidence of cases of type 4 streptococcal pharyngitis, type 4 "contact" carriers, and the carrier rate of miscellaneous Group A streptococci are presented in Figure 1.

No type 4 carriers were present in the community in February 1939 when the first 4 cases of pharyngitis due to this type appeared. The incidence of type 4 "contact" carriers rose

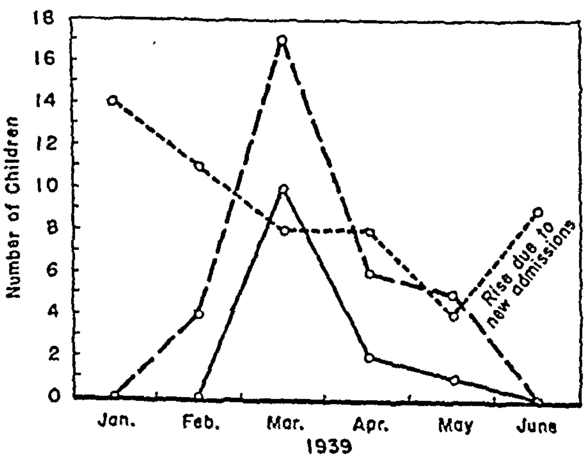

Fig. 1. Monthly Incidence of 32 Cases of Type 4 Pharyngitis, Type 4 Carriers, and Carriers of Miscellaneous Group A Streptococci

ʘ— — — Type 4 cases
ʘ——— Type 4 "contact" carriers
ʘ- - - - - "Healthy" carriers of miscellaneous Group A streptococci

sharply in March at the time when the greatest number of cases occurred and fell rapidly as the incidence of infections decreased. The carrier rate of other types of Group A streptococci fell gradually during the course of the outbreak.

In addition to the cases of upper respiratory infections due to type 4 streptococcus during March, 6 children developed pharyngitis due to Group A streptococci of undetermined type but with similar cultural characteristics. It was thought possible, therefore, that they might belong to one serological type. Attempts to produce agglutinating and precipitating sera by immunizing rabbits with one of these cultures were, however, unsuccessful and the serological identity of these 6 strains was not proved.

The remaining 56 children in the institution escaped streptococcal pharyngitis and did not become "contact" carriers of type 4 streptococcus.

2. *Outbreak of streptococcal pharyngitis due to type 27, October 1939 to May 1940*

Group A streptococcus carriers in the community on November 1, 1939. Sixty-seven of the 108 patients, followed during this winter, were "new" and 41, "old." Twenty-one of the 67 "new" children were "healthy" carriers of Group A streptococci. The following types were represented: types 2, 3, 32, 33, and 39, and 15 strains of undetermined types.

By November 1, only 6 of the 21 "healthy" carriers were still harboring streptococci: one child was carrying type 2, one type 39, and 4, streptococci of undetermined types.

Between September 11 and November 1, 2 "new" and 4 "old" children became carriers of type 33 streptococcus, a strain introduced by a "new" boy admitted in June 1939. During the course of 4 months, this type became disseminated among 22 children without, except in one instance, causing symptoms.

In addition to the carriers of type 33 streptococcus, 5 of the "old" children were carrying type 4 streptococcous, the epidemic strain of the previous year. One "old" girl carried type 29 streptococcus until the middle of January. Neither of these two types showed a tendency to spread to "new" children.

Outbreak of pharyngitis due to type 27 strepto-

coccus, November 2, 1939, to April 19, 1940. On October 31, a girl (G. C.) whose throat cultures had been consistently negative for Group A streptococci for 4 months, was sent to another hospital for tonsillectomy. She was readmitted on November 3, 2 days after operation, and had fever for 5 days after her return. Her throat cultures showed numerous type 27 streptococci, a type not previously present in the community. Another child, who had a tonsillectomy at the same hospital at the same time and who shared the same cubicle, failed to contract this infection.

Two weeks after the readmission of G. C., 3 other girls on the same ward developed pharyngitis due to type 27 streptococcus. During the next 6 months, 36 girls contracted this infection and 12 girls become "contact" carriers of type 27. In spite of the fact that 10 boys became "contact" carriers of the epidemic type, only 3 boys developed pharyngitis.

The monthly incidence of cases of type 27 streptococcal pharyngitis, type 27 "contact" carriers, type 33 streptococcus carriers, and carriers of miscellaneous Group A streptococci are presented in Figure 2.

The incidence of "contact" carriers of the epidemic-inducing type rose slowly compared with the incidence of cases: one child became a carrier in November when 5 cases appeared, and 2 in December when 8 further cases developed. The highest incidence of both "contact" carriers and cases occurred in January and then declined simultaneously. The carrier rate of type 33 streptococcus reached its peak in November. During December and January, both the miscellaneous Group A streptococcus carrier rate and that of type 33 were falling.

The remaining 46 children in the institution escaped streptococcal pharyngitis and did not become "contact" carriers of type 27 streptococcus.

3. *Outbreak of streptococcal pharyngitis due to type 15, October 1940 to February 1941*

Group A streptococcus carriers in the community during the fall of 1940. Seventy of the 108 patients followed during this winter were "new" and 38 "old." Nineteen of the 70 "new" children were "healthy" carriers of Group A streptococci. The following types were repre-

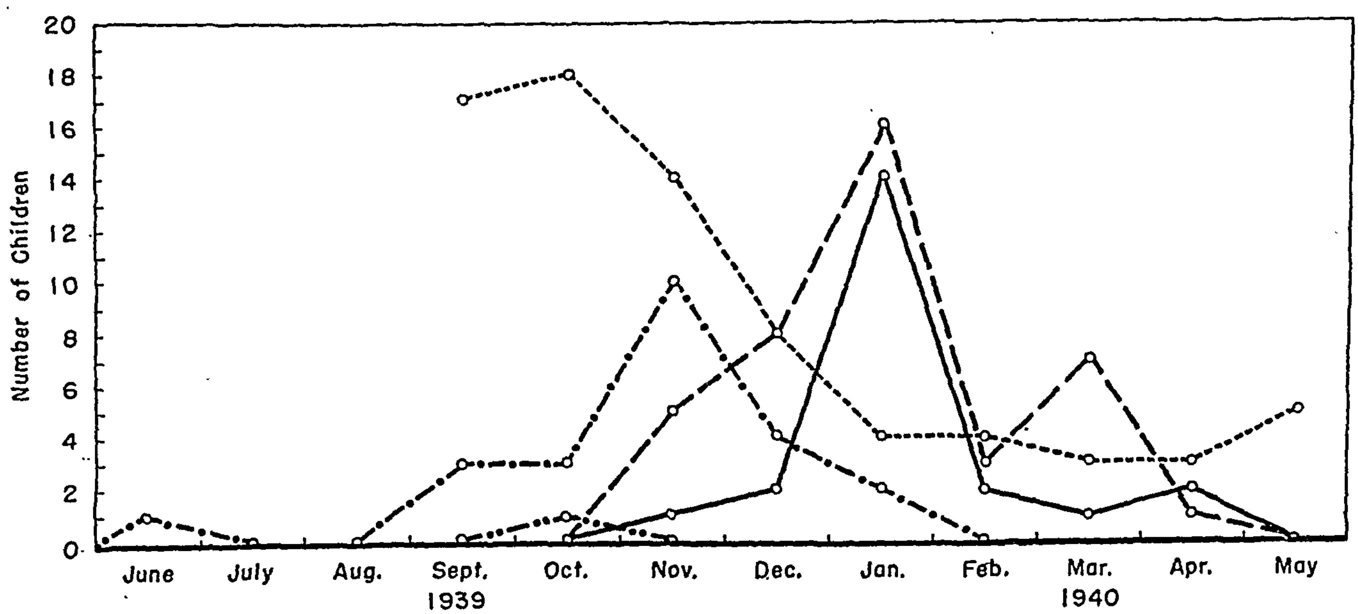

FIG. 2. MONTHLY INCIDENCE OF 40 CASES OF TYPE 27 PHARYNGITIS, TYPE 27 CARRIERS, TYPE 33 CARRIERS, AND CARRIERS OF MISCELLANEOUS GROUP A STREPTOCOCCI

⊙— — — Type 27 cases
⊙———— Type 27 "contact" carriers
⊙-·-·-·- Type 33 "contact" carriers
⊙- - - - - "Healthy" carriers of miscellaneous Group A streptococci
⊙-··-··- Type 33 case

sented: types 2, 6, 12, 13, 17, 26, 27, 33, 34, and 38, and 4 strains of undetermined types. One boy carried both type 6 and type 18.

Sporadic cases of streptococcal upper respiratory infections occurring in the fall of 1940. Eleven cases due to 3 different types of streptococci, types 6, 25, and 27, were observed during the fall of 1940. Between the middle of September and November 1st, 7 cases of type 27 pharyngitis occurred among the 70 "new" children who had not been in the institution the previous year when this type was epidemic. The upper respiratory symptoms in these 7 children were milder than those observed during the outbreak of the previous winter. The cultural characteristics of the type 27 streptococci, isolated from these cases, also differed from those of the strains, isolated during the previous epidemic: the capsules in India Ink preparations were smaller and the colonies less mucoid. One "new" child was found to be a type 27 carrier on admission. In September, 17 "old" children were still carrying type 27, so that there were many possible sources of this strain to account for these 7 cases. One "new" girl became a "contact" carrier of type 27, and harbored this strain for 6 months.

A single case of infection due to type 25 streptococcus occurred in September in a "new" boy, 2 months after admission. There were no known carriers of type 25 in the community at this time and the source of this strain was not determined. No other cases of infection due to type 25 developed; but 2 boys subsequently became temporary "contact" carriers of this type.

Two cases of type 6 streptococcal pharyngitis developed on one boys' ward where there were 2 "healthy" carriers of this type. The first case occurred in August and the second in September. In October, a third case due to type 6 occurred in a girl. Because the infection had jumped to one girls' ward, it was thought that an epidemic due to type 6 might be starting. Beginning October 18, therefore, daily prophylactic doses of sulfanilamide were given to half of the children in the institution. However, no further cases of pharyngitis due to type 6 streptococcus developed.

By the end of October, 17 of the 19 "new" children who were "healthy" carriers ceased to harbor hemolytic streptococci. The throat cultures of the boy carrying types 6 and 18 remained intermittently positive for both types for 8 months and another child carried streptococci of undetermined type for more than one year. Among the "old" children, one boy harbored type 34 streptococcus for 10 months. Four children were chronic "post-infection" carriers of type 27, the epidemic-inducing streptococcus of the previous year. Other carriers in the community at this time were the result of the 11 sporadic streptococcal infections which occurred from September until the end of October: 5 children were carrying type 27 streptococcus; 2, type 6; and one, type 25.

Outbreak of pharyngitis due to type 15 streptococcus, October 22, 1940 to January 29, 1941

Control group (i.e., *children not receiving sulfanilimide*). On October 17, 1940, a girl was admitted whose throat culture showed a single colony of type 34 streptococcus. A second culture, taken on the next day, showed no hemolytic streptococci. Five days after admission, a routine throat culture showed many type 15 streptococci but no type 34. The source of the type 15 strain was not determined. Although throat cultures obtained from this child continued to show numerous colonies of type 15 streptococcus, she showed no clinical or laboratory evidence of infection. She was, therefore, considered to be a carrier.

A series of cases due to type 15, which had not been previously present, developed first among the girls and then spread to the boys. During the next 3 months, among the 54 children who were not receiving sulfanilamide, 21 girls and 9 boys contracted pharyngitis due to this type. One other child became a "contact" carrier of type 15 streptococcus.

Sulfanilamide treated group. In spite of a blood level of 2 mgm. per cent of sulfanilamide, one child developed pharyngitis due to type 15 streptococcus. Ten children who were receiving prophylactic sulfanilamide became "contact" carriers of the epidemic-inducing type.

The monthly incidence of 11 sporadic cases of streptococcal upper respiratory infections due to types 6, 25, and 27, the outbreak of 31 cases of pharyngitis due to type 15, the carrier rates of types 6, 15, 27, and miscellaneous Group A

streptococci in the untreated and sulfanilamide treated groups are presented in Figure 3.

There was only one type 15 streptococcus carrier in the community in October when the first 3 cases of type 15 streptococcal pharyngitis appeared. In November, when 8 further cases among the children in the control group developed, 4 children receiving sulfanilamide became "contact" carriers. In December, when the outbreak reached its peak and 12 untreated children contracted the infection, one child in the sulfanilamide group developed pharyngitis due to type 15 streptococcus and one became a "contact" carrier. In January, the number of cases appearing in the control group declined to 7. No further cases developed among the children receiving sulfanilamide, but 2 more children became "contact" carriers. The carrier rate of type 27 streptococcus and of miscellaneous Group A streptococci decreased at approximately the same rate in control and sulfanilamide-treated groups.

In February, the throat cultures of 37, or 34 per cent, of the 108 children were positive for type 15 streptococcus. In spite of this large number of possible sources of infection, no new cases developed among the 24 children in the control group who had previously escaped infection.

4. *Outbreak of streptococcal pharyngitis due to type 36, October 1941 to March 1942*

Group A streptococcus carriers in the community during the fall of 1941. The group followed this winter included 73 "new" and 35 "old" patients. During September, 11 "new" children were "healthy" carriers of Group A streptococci. The following types were represented: types 1, 2, 4, 5, 14, 18, and 25, and 3 strains of undetermined types. (Two children carried the same type, type 2.)

In July, a "new" girl contracted mild pharyngitis due to type 35 streptococcus, 3 weeks after admission, and then carried this type for one year. In August and September, 3 other children developed upper respiratory infections due to type 35 and 2 girls became "contact" carriers. Three "old" children were still carrying type 15 streptococcus, the epidemic strain of the previous year. Thus, during September, 17 of 108 chil-

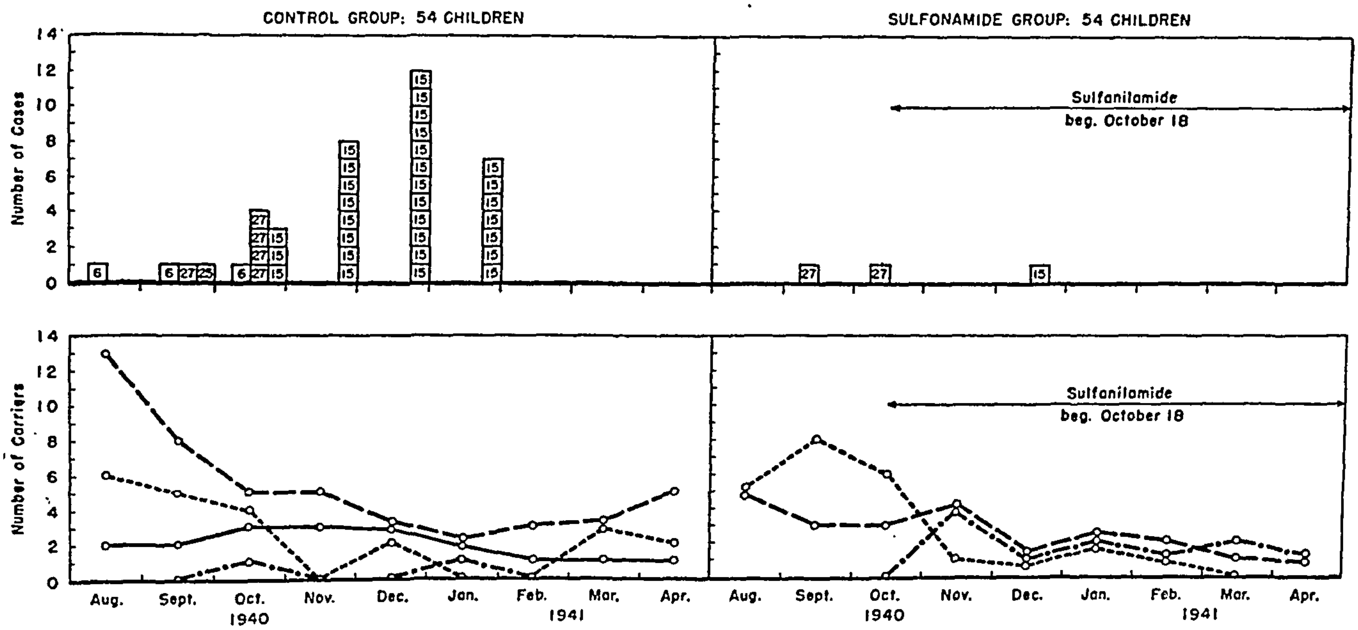

FIG. 3. MONTHLY INCIDENCE OF 10 SPORADIC CASES OF PHARYNGITIS DUE TO TYPES 6, 25, AND 27, AND AN OUTBREAK OF 30 CASES OF PHARYNGITIS DUE TO TYPE 15 STREPTOCOCCUS

6	Type 6 cases	⊙———	Type 6 "post-infection" and "contact" carriers
25	Type 25 cases	⊙— — —	Type 27 "post-infection" and "contact" carriers
27	Type 27 cases	⊙-·-·-·-	Type 15 "contact" carriers
15	Type 15 cases	⊙- - - - -	"Healthy" carriers of miscellaneous Group A streptococci

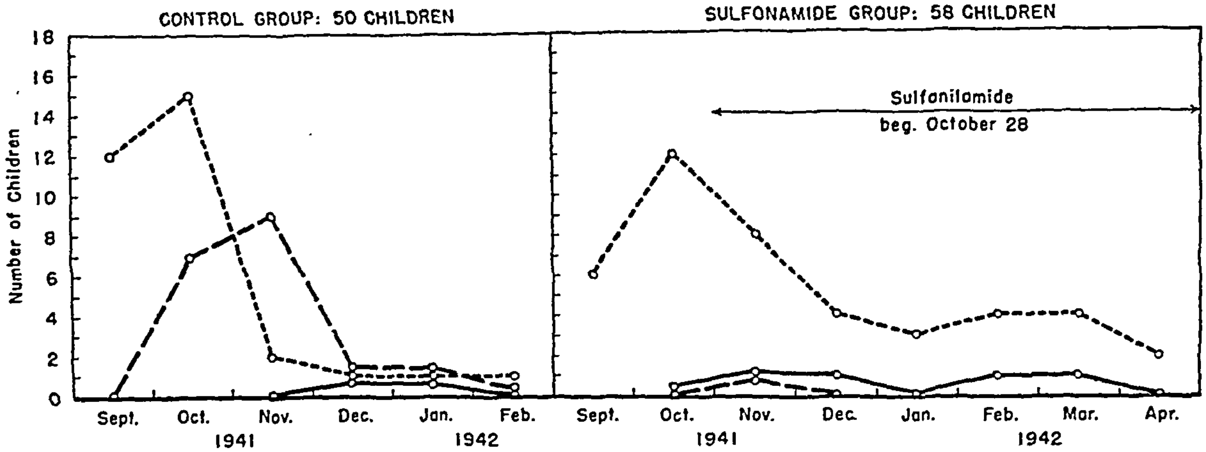

FIG. 4. MONTHLY INCIDENCE OF 18 CASES OF TYPE 36 PHARYNGITIS, TYPE 36 CARRIERS, AND CARRIERS OF MISCELLANEOUS GROUP A STREPTOCOCCI

⊙— — — Type 36 cases
⊙——— Type 36 "contact" carriers
⊙- - - - - "Healthy" carriers of miscellaneous Group A streptococci

dren were Group A streptococcus carriers. In October, the number of carriers rose to 27, due to the admission of "new" children who were carriers and to intermittent carriers whose throat cultures previously had shown streptococci but were negative in September. During November, the carrier rate dropped sharply and decreased still further in December and January.

Outbreak of pharyngitis due to type 36, October 26, 1941 to January 21, 1942. On October 21, a boy (H. B.) with slight coryza was admitted. His admission throat culture contained type 36 streptococcus, a type not previously present in the community. A series of cases of pharyngitis due to this strain developed, first among the boys and then spread to the girls. Beginning October 28, daily prophylactic doses of sulfanilamide or sulfadiazine were given to 58 children.

Control group. During the next 3 months, 9 boys and 8 girls among the 50 children serving as untreated controls contracted pharyngitis due to type 36. Two children became "contact" carriers of this type.

The remaining 30 children in the control group escaped streptococcal pharyngitis and did not become "contact" carriers of type 36.

Sulfonamide treated group. One boy developed pharyngitis due to type 36. The blood level of the drug at this time was less than 1 mgm. per cent. Four children in this group became "contact" carriers of type 36.

These data are shown in Figure 4.

In this outbreak, the first 6 cases of pharyngitis developed in October as the result of the introduction into the community of a boy with an upper respiratory infection. In November, 10 more children contracted the infection: 9 in the control and one in the sulfonamide-treated group. During this month, one child receiving sulfanilamide became a "contact" carrier of the epidemic-inducing type. The outbreak subsided quickly: only 2 more children who were not receiving sulfonamide prophylaxis developed pharyngitis, one in December and one in January. Three more "contact" carriers appeared among the children in the sulfanilamide-treated group, respectively in December, February, and March. Only 2 "contact" carriers were observed in the control group: one began to harbor type 36 streptococci in December and another in January. The carrier rate of miscellaneous Group A streptococci dropped more precipitously in the control than in the sulfonamide group.

Comparison of the length of the carrier state in "post-infection" and "contact" carriers

During the course of the 4 major outbreaks of streptococcal pharyngitis, 120 children contracted clinical infections and 39 became "contact" carriers. Septic complications occurred in only 2 of the 120 patients: one boy developed cervical adenitis and another chronic sinusitis. Since it is well known that individuals with purulent

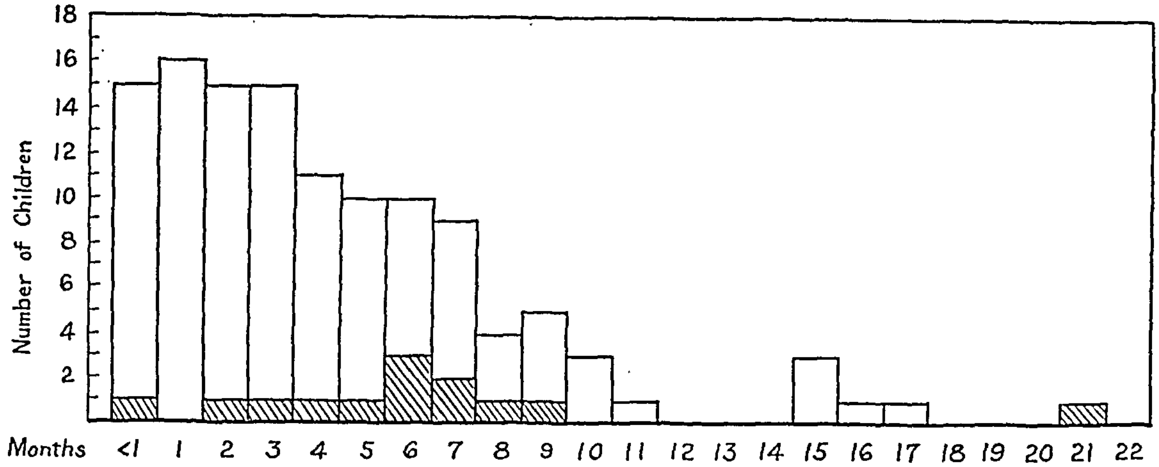

FIG. 5. ONE HUNDRED AND EIGHTEEN "POST-INFECTION" CARRIERS: LENGTH OF CARRIER STATE (4 MAJOR OUTBREAKS COMBINED)
Shaded areas = Individuals with tonsils

sequelae tend to harbor streptococci for long periods, these 2 boys were omitted from the comparison. Children in the sulfanilamide-treated groups, who became carriers of the epidemic-inducing strains, were not included among the 39 "contact" carriers, because it seemed possible that in the absence of sulfonamide prophylaxis these individuals might have developed clinical symptoms.

The data on the length of the carrier state in 118 "post-infection" and in 39 "contact" carriers is presented in Figures 5 and 6.

In spite of the fact that 105 of the 118 "post-infection" carriers had had their tonsils removed, many of these individuals harbored the epidemic-inducing strains for several months. The length of the carrier state in the "contact" was shorter than in the "post-infection" carriers: over 50 per cent of the former compared with 29 per cent of the latter carried streptococci for one month or less. Only 5 of the 39 "contact" carriers retained their tonsils.

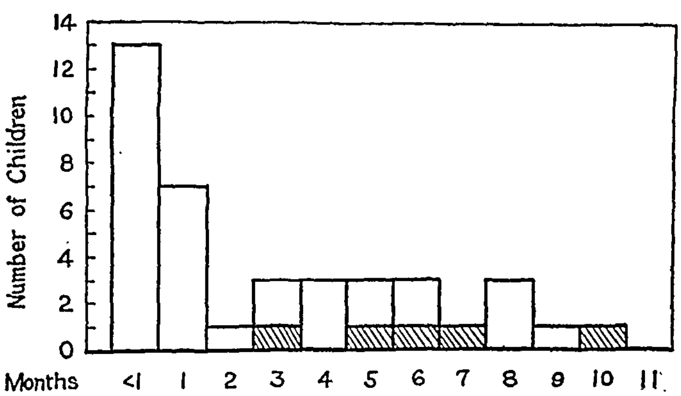

FIG. 6. THIRTY-NINE "CONTACT" CARRIERS: LENGTH OF CARRIER STATE (4 MAJOR OUTBREAKS COMBINED; "CONTACT" CARRIERS RECEIVING SULFONAMIDE PROPHYLAXIS NOT INCLUDED)
Symbols same as in Figure 5.

SUMMARY OF FINDINGS OBTAINED DURING THE 4 WINTERS 1938–42 (MAJOR OUTBREAKS)

During these 4 winters, when outbreaks of streptococcal pharyngitis due to Group A streptococci of single types (types 4, 27, 15, and 36, respectively) occurred, no rise in the carrier rate of the epidemic type preceded the appearance of cases. In the epidemics due to types 4 and 27, the incidence of "contact" carriers rose slowly and only reached a peak during the month when the greatest number of cases occurred. In the outbreaks due to type 15 and type 36, in each instance, only 2 children among those who were not receiving sulfonamide prophylaxis became "contact" carriers after the epidemic had started.

With the exception of the epidemic due to type 36 streptococcus, when a temporary rise occurred during the first month of the outbreak, the carrier rates of miscellaneous Group A streptococci were falling at the time that the epidemics began.

B. Minor outbreaks of streptococcal upper respiratory infections due to the dissemination of types present in the community

The 2 winters 1937–38 and 1942–43 are alike in that no major outbreaks occurred. Strains

carried by "healthy" carriers spread to other children who became "contact" carriers without developing evidence of infection, and subsequently, a few children developed pharyngitis due to these types.

1937–38. Weekly throat cultures were not taken routinely until January 1, 1938, and therefore no accurate data on the number of Group A carriers present in the community during the summer and fall of 1937 were available.

In May 1937, a boy carrying type 32 streptococcus was admitted. Five children became "contact" carriers of this type before the first case appeared the end of December. This infection spread slowly and did not reach a definite peak: during each of the 3 succeeding months (January, February, and March), 3 cases occurred, followed by one in April and another in May, making a total of 12. The upper respiratory symptoms associated with this infection were mild. From the beginning of January until April, 9 more children became contact carriers of type 32.

Group A streptococcus carriers in the community during the winter 1942–43

During this winter the group included 71 "new" and 37 "old" patients. Seventeen "new" children were Group A streptococcus carriers. The following types were represented: types 1, 2, 14, 17, 18, 19, 41, and 44, and 6 strains of undetermined types. By November 1, 7 of these 17 carriers no longer harbored hemolytic streptococci.

Type 19. Type 19, however, introduced by a boy admitted in July, showed a tendency to spread to other children. During the summer, 5 other boys became "contact" carriers of this type. In October, a girl developed pharyngitis due to this type; and one month later, a boy contracted this infection. Neither of these children developed rheumatic sequelae. No further cases of pharyngitis due to type 19 appeared although one "contact" carrier harbored this type until May and one "post-infection" carrier until June.

Four of the 37 "old" children were carriers of Group A streptococci. Two children carried type 36, the epidemic strain of the previous year. One "new" girl became a "contact" carrier of this strain in February and carried this type for 2 months. One child carried type 44, but this strain showed no tendency to spread to other children.

Type 37. One boy, admitted in November 1941, was an intermittent carrier of type 37 for more than one year. During the first winter of his stay, 1941–42, 2 children became temporary "contact" carriers of this type, but no cases of pharyngitis developed. During the second winter, 1942–43, 5 children again became "contact" carriers and 6 other children contracted upper respiratory infections due to this type. Two of the latter subsequently developed mild rheumatic manifestations.

SUMMARY OF FINDINGS OBTAINED DURING THE 2 WINTER SEASONS, 1937–38 AND 1942–43 (MINOR OUTBREAKS)

During both these winters, a relatively small number of cases of streptococcal pharyngitis occurred in the community. In each instance the infections were caused by the dissemination of types derived from known carriers who had been in the community for several months. These strains first spread to other children who became "contact" carriers. Subsequently, a few children developed pharyngitis due to these types. During 1937–38, 12 cases of pharyngitis were caused by type 32 streptococcus and during 1942–43, 2 cases by type 19 and 6 by type 37.

SUMMARY OF FINDINGS REGARDING "HEALTHY" OR ADMISSION CARRIERS

During the 5-year period, 1938–43, when the weekly incidence of Group A carriers was determined, 102 of the 348 "new" children were

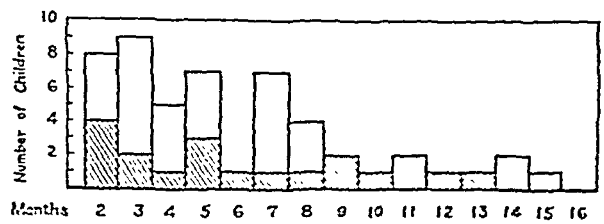

FIG. 7. FIFTY-ONE CHRONIC "HEALTHY" OR ADMISSION CARRIERS: LENGTH OF CARRIER STATE (33, OR 65 PER CENT, HAD HAD TONSILLECTOMIES)

Symbols same as in Figure 5.

"healthy" carriers of Group A streptococci. Fifty-one or 50 per cent, of the 102 "healthy" carriers harbored streptococci for 2 months or longer while under observation. The length of the carrier state in these 51 individuals, the majority of whom had had their tonsils removed, is presented in Figure 7.

DISCUSSION

The incidence of outbreaks and cases of streptococcal pharyngitis and of Group A streptococcus carriers, observed in groups of rheumatic children during 6 successive winters, has been described. The relation of these upper respiratory infections to rheumatic relapses has been previously reported and, except in the case of the current year, 1942–43, is not discussed.

During each of 4 winter seasons when a fairly large number of streptococcal upper respiratory infections due to single types of Group A streptococci occurred in the community, a rise in the carrier rate of the epidemic-inducing types did not precede the appearance of the epidemics. These strains were introduced from outside sources and were not derived from Group A streptococcus carriers who had been in the community for any length of time.

During each summer, a fairly large proportion of the children admitted were "healthy" carriers of Group A streptococci. The majority of these individuals had had tonsillectomies. In most instances, these strains did not spread to other children, and usually disappeared from throat cultures by the beginning of the winter season (*i.e.*, December 1). Some carriers, however, irrespective of whether or not their tonsils had been removed, continued to carry the same type for periods as long as 2 years, but, in spite of prolonged and intimate contact during the winter months, did not cause infection in other individuals.

Occasionally, however, types derived from carriers spread to other children who also became carriers; and subsequently, a few cases of pharyngitis due to these types appeared. In one instance, a single type of a Group A streptococcus (type 33) became widely disseminated but caused only one mild upper respiratory infection. Other types infected a small number of children and then failed to spread further.

It is well known that food-borne outbreaks of streptococcal upper respiratory infections are usually explosive and subside quickly. On the other hand, epidemics of streptococcal pharyngitis or scarlet fever, where the infection is transmitted from person to person, often last for weeks and months. This difference is undoubtedly related to the size of the infecting dose. Under ordinary conditions, only a small number of bacteria are transferred from one individual to another. It seems probable that, in many instances where individuals are in close contact, the number of streptococci necessary to cause symptoms may be acquired fractionally as suggested by Dudley. A considerable period of time may elapse before a sufficiently large dose is transferred, thus explaining the slow spread of the infection.

Aside from the size of the infecting dose, differences in susceptibility also play a part. In the presence of sporadic infections, when a few children in a ward develop streptoccal pharyngitis due to a single type and the rest remain uninfected, it seems probable that the latter group are relatively resistant to that particular type. Even during major outbreaks, when the number of "post-infection" and "contact" carriers in the community was large, many children escaped infection. At the present time, our knowledge of streptococcal immunity is very limited; but in our experience, children usually do not contract pharyngitis due to the same type more than once. These observations suggest the existence or development of type-specific immunity.

The possibility that the infectivity of different types or variants of the same type of streptococci may vary from season to season and from year to year must also be considered. During each of the 4 years when major outbreaks of streptococcal upper respiratory infections occurred, a few children carrying the epidemic type of the previous winter remained in the institution. In spite of the fact that large groups of "new" children were admitted during the summer and fall months, in only one instance did the epidemic type of the previous year induce a few cases of pharyngitis among the "new" children. During the autumn of 1940, 7 of the 70 newly admitted children developed mild upper respiratory infec-

tions due to type 27, the type prevalent in the institution during the previous winter. The streptococci isolated from these 7 patients had smaller capsules than those isolated during the original outbreak and the colonies were matt rather than mucoid. It is also well known that strains isolated at the end of an outbreak often give less well defined serological reactions than those isolated at the beginning. This evidence suggests that the streptococci themselves tend to change during the course of epidemics.

Some investigators believe that individuals do not become carriers of Group A streptococci except as the result of infection but our experience is not in accord with this view. In 2 of the major outbreaks, due, respectively, to type 15 and type 36 streptococci, only a few children became "contact" carriers of the epidemic-inducing strain. In the 2 outbreaks, due, respectively, to streptococci types 4 and 27, on the other hand, a large number of children were considered to be "contact" carriers. The spread of infection with types 4 and 27 was slower and the clinical symptoms less severe than with type 15 or type 36. Our observations suggest that the incidence of "contact" carriers varies inversely with the virulence of the infecting strain. Thus, in the case of type 33 which became widely disseminated during the autumn of 1939, 22 children became "contact" carriers and only one child developed mild upper respiratory symptoms due to this type. This strain formed small matt colonies and no capsule was demonstrable in India Ink preparations.

There is no doubt that the virulence, not only of different types of Group A streptococci but also of different strains of the same type, may vary widely. Individuals sometimes become chronic carriers of streptococci belonging to groups other than Group A, groups rarely pathogenic for human beings. It seems possible therefore that Group A streptococci of low virulence or those belonging to types to which the individual is resistant may exist as saprophytes on the mucous membranes of the nasopharynx. At the present time, in our opinion, the conclusion that all "contact" carriers are really the result of infection is open to question.

Our findings that outbreaks of streptococcal pharyngitis are not necessarily preceded by a rise in the carrier rate of the epidemic-inducing type are not in accord with those of most other investigators. The epidemics studied were small, but the data included the whole community and were not based on the selection of samples considered to be representative. The observations were limited to rheumatic children, and the susceptibility of the rheumatic individual to streptococcal infections may differ from that of the normal. The fact that, during each winter, the group of children was fairly isolated may also have influenced our findings. The majority of the infections were not severe; and none of the children developed scarlet fever. The possibility that some of our cases were so mild that these individuals might have been considered to be "healthy" carriers in communities with less medical supervision does not, however, explain the discrepancy between our observations and those of others. The symptoms developed by some of the patients at the beginning of each outbreak were sufficiently severe (temperatures of 102 to 104° F.) so that they would not have been overlooked. In our opinion, major outbreaks of streptococcal pharyngitis are usually not caused by chronic Group A carriers in the community. The greatest source of danger is the introduction into the group of an individual with an active streptococcal infection, due to a type not previously present.

SUMMARY

1. Major and minor outbreaks as well as sporadic cases of streptococcal pharyngitis occurring in groups of rheumatic children in a sanatorium during a 6-year period are described.

a. Major outbreaks were due to Group A streptococci of a single type not previously present and were not preceded by a rise in carrier rate.

b. Minor outbreaks were preceded by a slow spread from carriers to other individuals without at first causing infection. Subsequently, a small number of clinical cases due to these types developed.

c. Sporadic cases arose directly from carriers and were not preceded by a dissemination of the streptococci to healthy individuals.

2. The length of the carrier state was studied.

a. Twenty-nine per cent of the children admitted during the summer and fall months were carriers of Group A hemolytic streptococci. With few exceptions, these microorganisms did not spread to other individuals and disappeared after a few months.

b. The epidemic-inducing types of streptococci persisted longer in "post-infection" than in "contact" carriers.

c. The length of the carrier state was not related to the presence or absence of tonsils.

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THE OCCURRENCE OF BACTERIOSTATIC PROPERTIES IN THE BLOOD OF PATIENTS AFTER RECOVERY FROM STREPTOCOCCAL PHARYNGITIS[1]

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Although no definite proof is available, our observations covering 6 years suggest that repeated attacks of streptococcal pharyngitis occurring in the same individual are usually due to Group A streptococci of different types. Reinfection with the same type was never observed, suggesting that type-specific immunity may develop. At the present time, however, serological evidence of such immunity is meager.

REVIEW OF LITERATURE

Fothergill and Lium studied the bactericidal activity of the blood of 4 convalescent scarlet fever patients (1). Two of these patients recovered without developing complications; the other 2 developed otitis media, in one instance accompanied by mastoiditis, and in the other by adenitis. Only the 2 patients with purulent sequelae showed an increase in the bactericidal activity of the blood for the homologous streptococcus.

Hare reported the development of specific bactericidal properties in the blood of patients during the course of puerperal sepsis (2).

Spink and Keefer studied the streptococcidal power of defibrinated blood obtained from 30 erysipelas patients (3). An increase in bactericidal power for the homologous organism during the course of the disease was observed in one-third of the cases.

Chandler and Taussig, in a recent article, reported that the blood of 2 rheumatic children, who were carriers of Group A streptococci, were bactericidal for the homologous strains (4). The blood of 2 carriers of Group C streptococci was also found to contain homologous bactericidal antibodies.

Tillett (5) showed that sera obtained from patients acutely ill with a variety of infectious diseases, such as pneumonia, meningococcus meningitis, tuberculous pleurisy, as well as diseases due to streptococci, was highly bactericidal for certain strains of streptococci. This property disappeared as the patients recovered and was not considered to represent a specific immune response.

Swift and Hodge described the occurrence of type-specific anti-M precipitins in the sera of patients following streptococcal infections (6). Positive reactions were obtained, both in patients who developed rheumatic sequelae and in those who did not.

Walker (7) has reported the presence of type-specific agglutinins for the homologous streptococcus, demonstrable by the slide agglutination technique of Griffith, in the sera of 6 of 22 patients. The 6 positive reactions were obtained with sera of individuals recovering from purulent streptococcal infections (otitis media, empyema, and paronychia accompanied by axillary adenitis).

Rantz *et al.* reported the development of agglutinins for the homologous types, demonstrable by the slide agglutination technique, in 13 of 22 cases of scarlet fever (8).

Diefendorf (9) was able to demonstrate mouse protective antibodies in the sera of 10 of 14 patients. Six of these 10 sera were obtained from patients recovering from scarlet fever and 4 from rheumatic subjects during the fourth week of the rheumatic attack.

MATERIAL

Outbreak of streptococcal pharyngitis. As previously reported, an outbreak of pharyngitis, due to a Group A streptococcus of a single type, type 36, occurred in this institution during the winter of 1941–1942 (10, 11). Six cases occurred in October, 9 in November, followed by 2 in December and one in January (making a total of 18 cases). Sera for the determination of antistreptolysin O titer, slide agglutination, and anti-M precipitin reactions were collected at frequent intervals, following the upper

[1] This study was aided by a grant from The Dazian Foundation.

TABLE I—*Continued*

Case No.	Name Hosp. No.	Age	Pharyngitis: Date	Pharyngitis: Severity	Rise in anti-streptolysin 0 titer	Interval between infection and tests	Dates of tests	Results: Patient after recovery	Results: Children who escaped type 36 pharyngitis: Control No. 1	Results: Children who escaped type 36 pharyngitis: Control No. 2
			9 PATIENTS WHO DEVELOPED RHEUMATIC MANIFESTATIONS—*Continued*							
		years			*units*	*months*				
6	C. D. 3671	11	November 4, 1941	0	125 to 500	5	April 7, 1942	5/14	9/∞	
				(Throat culture positive)		6	May 7, 1942	5/11	5/∞	
						17	April 28, 1943	3/243	5/∞	2/many
7	H. L. 3703	7	November 6, 1941	++	125 to 400	5	April 16, 1942	0/1	7/258	
						$6\frac{1}{2}$	May 28, 1942	2/0	5/275	2/416
8	M. M. 3596	11	December 7, 1941	+	200 to 600	$3\frac{1}{2}$	March 23, 1942	24/13	36/∞	33/∞
						5	May 3, 1942	5/0	5/∞	
						6	June 4, 1942	0/2	2/166	0/60
9	E. J. 3655	12	November 16, 1941	±	125 to 165	$4\frac{1}{2}$	March 31, 1942	3/31	7/299	2/110
						5	April 21, 1942	6/11	4/246	4/204
						6	May 14, 1942	1/6	0/135	2/121
			7 PATIENTS WHO ESCAPED RHEUMATIC SEQUELAE							
10	A. H. 3673	8	October 26, 1941	+++ (adenitis)	13 to 165	$5\frac{1}{2}$	April 10, 1942	61/95	60/∞	67/∞
						7	May 20, 1942	4/8	3/∞	2/many
						10	August 21, 1942	7/23	7/∞	4/∞

TABLE I—*Continued*

Case No.	Name Hosp. No.	Age	Pharyngitis: Date	Pharyngitis: Severity	Rise in anti-streptolysin 0 titer	Interval between infection and tests	Dates of tests	Results: Patient after recovery	Results: Children who escaped type 36 pharyngitis: Control No. 1	Results: Children who escaped type 36 pharyngitis: Control No. 2
7 PATIENTS WHO ESCAPED RHEUMATIC SEQUELAE—*Continued*										
		years			*units*	*months*				
11	D. G. 3624	7	October 31, 1941	++	50 to 100	5	March 27, 1942	4/0	6/137	4/92
						7	May 26, 1942	1/2	2/127	2/98
						12	October 30, 1942	5/0	3/many	1/277
12	J. O'L. 3626	11	November 4, 1941	++	32 to 80	5	April 3, 1942	5/4	3/107	3/106
						7½	June 25, 1942	0/3	0/∞	1/many
13	M. C. 3635	8	November 5, 1941	++	60 to 100	5½	April 21, 1942	1/1	4/246	4/204
						7	June 12, 1942	3/0	0/many	1/many
						13	December 15, 1942	3/39	2/many	6/many
14	M. N. 3682	8	November 9, 1941	++	no change	5	April 7, 1942	7/0	8/∞	9/∞
						6½	May 28, 1942	4/3	1/216	1/190
						8½	August 27, 1942	0/16	0/383	0/407
						14	January 26, 1943	8/11	6/many	
						16	March 25, 1943	7/many	2/∞	5/∞
15	K. W. 3734	11	November 14, 1941	0 (Throat culture positive)	100 to 165	5½	April 23, 1942	0/10	0/258	0/70
						6½	May 28, 1942	2/8	2/416	5/275
16	C. B. 3634	13	December 31, 1941	0 (Throat culture positive)	60 to 100	4	April 21, 1942	2/23	4/246	4/204
						5	May 24, 1942	2/[illegible]	0/[illegible]	0/[illegible]

one child known to have no bacteriostatic activity against type 36 streptococcus was used throughout the experiment. Five-tenths cc. of the serum to be tested was added to 0.9 cc. of this boy's blood. The results are presented in Table II.

Unfortunately, only small amounts of sera obtained before the attack of pharyngitis were available and the tests therefore could not be repeated. Although the bacteriostatic activity of the sera was weak, in 4 of the 6 cases, a definite difference between the sera taken before and after infection was apparent.

Specificity of the bacteriostatic activity of the blood of patients, following recovery from streptococcal pharyngitis

Strains of heterologous types (types 6, 15, 19, 26, and 39), isolated from patients with acute pharyngitis, were included in many of the tests. It was shown by Todd (17) that the virulence of streptococci can be judged by their ability to grow in the blood of normal individuals. Although the heterologous types were recently isolated, most of these strains appeared to be of low virulence and failed to grow in human blood unless a fairly large number of bacteria were inoculated. With the homologous strain, type 36, a considerable variation in the size of the inoculum did not alter the results. With the heterologous types, the bacteriostatic activity of the blood of many of the children varied with the number of bacteria inoculated. In many instances, when a small inoculum was used, the blood appeared to be bacteriostatic, whereas, with a larger inoculum, the bacteria were able to multiply. Furthermore, the findings with heterologous types were often equivocal because no definite contrast between the child who had recovered from the type 36 streptococcal pharyngitis and the children chosen to serve as controls was obtained. In most instances, however, the bacteriostatic effect was more marked against the homologous type 36 than against heterologous types.

Absorption. It was thought of interest to determine whether the bacteriostatic activity of sera obtained from children whose whole blood inhibited the growth of type 36 streptococcus could be reduced by absorption with the homologous strain. The inactivated serum in each instance was divided into 3 parts: one part was not absorbed, one was absorbed with the homologous strain, and one with a streptococcus of a heterologous type. Two parts of serum were mixed with one part of heat-killed bacterial sediment, and incubated at 37° C. for 30 minutes. The mixture was allowed to stand overnight in the ice box, centrifuged the following morning, and the clear serum pipetted off. Five-tenths cc. of each of these sera was added to 0.9 cc. of whole heparinized blood, obtained from a child known to have no bacteriostatic action against type 36 streptococcus.

The indirect method of doing bacteriostatic tests, using serum and blood from a control, usually gave less definite results than those obtained with whole blood of children following recovery from type 36 pharyngitis. In most instances, however, the bacteriostatic action of serum absorbed with the homologous type was reduced, as compared with the unabsorbed serum or that absorbed with a heterologous type. A protocol illustrating these findings is presented in Table III. The bacteriostatic effect as shown in this table is most marked with serum obtained from Case 5 although this child was bled 16 months after the attack of pharyngitis. In all 3 of these cases, the bacteriostatic action of the serum was definitely reduced after absorption with the homologous type.

Comparison of the bacteriostatic activity of the blood of children receiving prophylactic doses of sulfanilamide with that of children after recovery from pharyngitis

In a number of experiments, the blood of patients receiving daily prophylactic doses of sulfanilamide was included. No para-aminobenzoic acid was added. It was found that, in most instances, the blood of patients containing concentrations of free sulfanilamide, varying from 1.2 to 2 mgm. per cent, was less bacteriostatic for type 36 streptococcus than that of children who had recovered from pharyngitis due to this microorganism.

Is type 36 streptococcus peculiarly susceptible to the bactericidal action of serum described by Tillett?

It was noted by Tillett that different strains of streptococci varied greatly in their suscepti-

TABLE II

Comparison of the bacteriostatic activity of serum obtained before and after infection with type 36 streptococcus

Case No.	Date of pharyngitis	Inactivated serum		Blood from control, C.B.	Result
		Date	Amount		
			cc.	*cc.*	
1	October 27, 1941	October 16, 1941	0.5	0.9	$\frac{6}{\text{many}}$
		March 27, 1942	0.5	0.9	$\frac{7}{26}$
7	November 6, 1941	July 2, 1941 October 25, 1941	0.5	0.9	$\frac{8}{\text{many}}$
		March 20, 1942	0.5	0.9	$\frac{7}{55}$
10	October 26, 1941	July 30, 1941	0.5	0.9	$\frac{5}{\text{many}}$
		January 12, 1942	0.5	0.9	$\frac{7}{83}$
12	November 4, 1941	August 27, 1941	0.5	0.9	$\frac{8}{\text{many}}$
		January 6, 1942	0.5	0.9	$\frac{13}{304}$
14	November 9, 1941	October 6, 1941	0.5	0.9	$\frac{8}{\text{many}}$
		December 16, 1941	0.5	0.9	$\frac{4}{\text{many}}$
16	December 31, 1941	December 27, 1941	0.5	0.9	$\frac{5}{\text{many}}$
		February 12, 1942	0.5	0.9	$\frac{7}{83}$
Control C. B.			(0.5 saline)		$\frac{5}{\infty}$

bility to the non-specific bactericidal action of sera obtained from febrile patients (5). Although all the children whose blood was used in the bacteriostatic tests were afebrile and had no complaints or symptoms, in a few experiments, the bacteriostatic action of fresh serum was compared with that of whole blood obtained from the same individual. In spite of the fact that the blood was definitely bacteriostatic, it was found that type 36 streptococcus multiplied freely in the serum. The bactericidal action of fresh serum obtained from one patient with active rheumatic fever, whose temperature ranged between 101.8 to 103° F. on the day of the test, was also tried with type 36 streptococcus. No bactericidal effect was noted. Thus, no evidence

TABLE III

Effect of absorption with the homologous type and a heterologous type of sera, obtained from children after recovery from streptococcus type 36 pharyngitis

Case No.	Date of pharyngitis	Date serum obtained	Inactivated serum		Whole blood from control J.M.	Result
			Absorption	Amount		
				cc.	*cc.*	
5	November 4, 1941	March 25, 1943	Unabsorbed	0.5	0.9	3/57
			Absorbed with homologous type 36	0.5	0.9	3/many
			Absorbed with heterologous type 11	0.5	0.5	3/59
6	November 4, 1941	Pooled sera obtained between December 9, 1941 and July 2, 1942	Unabsorbed	0.5	0.9	4/89
			Absorbed with homologous type 36	0.5	0.9	0/many
			Absorbed with heterologous type 11	0.5	0.9	1/117
11	October 31, 1941	March 30, 1943	Unabsorbed	0.5	0.9	3/341
			Absorbed with homologous type 36	0.5	0.9	3/many
			Absorbed with heterologous type 11	0.5	0.9	2/146
		Serum from control S. A.	Unabsorbed	0.5	0.9	3/many
		Serum from control M. H.	Unabsorbed	0.5	0.9	0/many
					1.4	3/many

was obtained to suggest that the phenomenon described by Tillett played a part in our findings.

Is the bacteriostatic action of the blood demonstrable in children following recovery from upper respiratory infections due to other types of Group A streptococci?

Type 15 streptococcus. Blood was obtained from 5 children, who had had pharyngitis, due to type 15 streptococcus, 15 to 18 months after the upper respiratory infection. No definite bacteriostatic effect against the homologous type was demonstrable.

Type 19 streptococcus. The blood of 2 children, who had had upper respiratory infections associated with type 19 streptococcus during the winter of 1942, was tested with the homologous type and with 2 heterologous types. In one of these patients, the bacteriostatic action of the blood for type 19 streptococcus had been determined be-

fore the infection occurred, and in the other, the first test was done 3 weeks after the attack of pharyngitis. Further tests were performed with the blood of both children, at frequent intervals for periods of 5 to 6 months. In some instances, the blood was set up in duplicate and inoculated with varying numbers of bacteria. In one experiment, the bacteriostatic activity of whole blood was compared with that of freshly obtained serum.

The data presented in Table IV indicate that the bacteriostatic action of the blood in the case of these 2 children became demonstrable only 2 to 3 months after infection. At this time, the serum, in contrast to the whole blood, showed no bactericidal or bacteriostatic effect. The blood of neither of these 2 individuals inhibited the growth of 2 heterologous types, 36 and 37. The bacteriostatic action of J. C.'s blood for the homologous type 19 streptococcus was slightly

TABLE IV

Bacteriostatic tests using the homologous and heterologous types with blood and serum obtained from 2 children who had type 19 streptococcal pharyngitis

Name	Pharyngitis		Rise in antistreptolysin O titer	Interval between infection and tests	Dates of tests	Strains of streptococci	Results				
							Patient		Children who escaped type 19 pharyngitis		
									Control 1		Control 2
	Date	Severity					Whole blood	Fresh serum	Whole blood	Fresh serum	Whole blood
							0.9 cc.	0.9 cc.	0.9 cc.	0.9 cc.	0.9 cc.
J. C.	October 26, 1942	+++	No change	3 weeks	November 18, 1942	Homol.* type 19	2/many		1/many		3/267
				5 weeks	December 4, 1942	Homol. type 19	0/many		0/many		1/327
				3 months	January 29, 1943	Homol. type 19	3/1	3/many	1/many	3/many	
				3½ months	February 19, 1943	Homol. type 19	4/27		3/many		6/∞
						Heter.** type 37	1/208		0/many		3/many
				6 months	April 28, 1943	Homol. type 19	1/7		1/many		3/∞
						Heter. type 36	0/many		5/480		2/many
H. R.	December 1, 1942	++	No change	Before infection	November 14, 1942	Homol. type 19	1/many		0/113		0/66
				2 months	January 29, 1943	Homol. type 19	3/50	2/many	1/many	3/many	
				2½ months	February 19, 1943	Homol. type 19	0/46		3/many		6/∞
						Heter. type 37	5/∞		0/many		3/many
				5 months	April 28, 1943	Homol. type 19	1/58		1/many		1/∞
						Heter. type 36	1/many		5/4[illegible]0		2/many

* Homol. = Homologous.

** Heter. = Heterologous.

more marked than that of H. R. The clinical symptoms were also somewhat more severe in the former patient.

Limitations of bacteriostatic tests

Different observers have used various methods of doing bactericidal tests. Most investigators have emphasized the difficulties and numerous sources of error inherent in this technique.

The modification of the method employed by us differs from that used by other observers in that we obtained merely a bacteriostatic and not a bactericidal effect. The number of bacteria inoculated was small and the period of incubation short. It is only by comparing the results obtained with the blood of suitable controls that our findings are of interest.

DISCUSSION

The development of bacteriostatic properties in the blood of patients following recovery from streptococcal pharyngitis due to a single type of Group A hemolytic streptococcus, type 36, is reported. This property persisted for many months and in some instances was still demonstrable a year after the upper respiratory infection. It developed irrespective of whether the symptoms caused by the pharyngitis were mild or severe and occurred both in patients who escaped and those who developed rheumatic sequelae.

At the present time, the majority of investigators agree that rheumatic recurrences usually follow in the wake of streptococcal pharyngitis. One of the major concerns of physicians who have rheumatic patients under their care is the prevention of this kind of upper respiratory infection. It has been shown by several observers that streptococcal pharyngitis and rheumatic relapses can be prevented by the prophylactic administration of sulfanilamide (18 to 20, 4, 10). There are, however, many objections to prolonged sulfonamide prophylaxis and it seems worthwhile therefore to investigate the possibility that type specific immunity may develop.

It is noteworthy that in outbreaks of streptococcal pharyngitis or scarlet fever due to a single type of Group A hemolytic streptococci, a large proportion of the individuals exposed escape infection. At the present time, the nature of this resistance is not understood. It may indicate a previous infection with the same type or with types so closely related antigenically than an overlapping immunity is produced. If type-specific immunity does develop, it may be cellular and bear no relation to humoral antibodies. On the other hand, it is entirely possible that the failure to contract infection depends on unknown non-specific factors.

During the course of the outbreak of pharyngitis, due to type 36 streptococcus, the failure of certain children to contract this infection, in spite of exposure, was striking. It was thought that if the resistance of these individuals was due to a previous infection with this type of streptococcus, bacteriostatic properties might still be demonstrable in their blood. The results of these tests, however, were entirely negative. It may be that, after a lapse of time, bacteriostatic activity is no longer demonstrable in the blood and that these humoral antibodies represent a temporary expression of a more permanent cellular immunity.

Bacteriostatic tests failed to elucidate the nature of the resistance to streptococcal pharyngitis of individuals who escaped infection. The fact that this property was demonstrable in the blood of patients recovering from pharyngitis due to Group A of a single type, however, gives us another method for studying the development of type-specific immunity following streptococcal upper respiratory infections. By means of this technique, it may be possible to answer the much disputed question: Are so-called "contact" carriers, who during outbreaks of streptococcal pharyngitis acquire the epidemic-inducing strain, really cases of subclinical infection?

SUMMARY

The development of bacteriostatic properties in the blood of children, after recovery from upper respiratory infections due to Group A streptococci of a single type, is reported.

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ON THE PATHOGENESIS OF RENAL FAILURE ASSOCIATED WITH MULTIPLE MYELOMA. ELECTROPHORETIC AND CHEMICAL ANALYSIS OF PROTEIN IN URINE AND BLOOD SERUM[1]

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One of the factors concerned in the pathogenesis of renal insufficiency in many cases of Bright's disease is mechanical obstruction of glomeruli and tubules by accumulations of fibrin and other more abundant protein coagula. It has been shown by means of chemical and electrophoretic analysis of protein excreted in the urine of such cases that there is a close correlation between the concentration of globulin and total protein, and the rate at which protein coagula collect within the kidney (1 to 3). The evidence suggests, further, that the hyaline coagula, not identifiable as fibrin, consist chiefly of precipitated globulins.

The example of multiple myeloma, Bence-Jones proteinuria, and renal insufficiency described here represents a type of Bright's disease in which renal insufficiency depends chiefly on obstruction of tubules by precipitation of Bence-Jones protein. The blood serum of this case contained a fraction comprising 24.6 per cent of the total serum protein, and it moved electrophoretically like a beta globulin. The protein in the urine salted-out like a globulin, moved electrophoretically like a beta globulin, and exhibited the solubility characteristics typical of a Bence-Jones protein.

CASE REPORT

M. T., History No. 232997, a white man, 45 years old, was first examined on June 30, 1941, and died on January 25, 1942.

History. Six months before examination, the patient noticed weakness and fatigue. He broke a rib by merely leaning against a dull instrument with which he was working. During the next few months, there appeared dyspnea, dizziness, occasional numbness and tingling in the legs, nocturia on occasions, blurring of vision, and vomiting.

Physical examination. He was somewhat obese, and was very pale. The blood pressure varied from 130/80 to 170/80 (3 determinations in 6 months). The temperature was elevated nearly every day (99° to 100° usually). The tip of the spleen was palpable. *Blood:* The red blood cell count was 1,200,000; the hemoglobin was 30 per cent (4.2 grams); the white blood cell count was 4,200; differential was normal, except for 9 per cent myelocytes. *Serum protein* was 6.4 to 7.0 grams per cent (5 determinations in 5 months). Fractionation of the serum by Howe's sulfate method showed in July, albumin 4.76 grams, globulin 1.66 grams (A/G 74/26); and in January, albumin 4.94 grams, globulin 1.93 grams (A/G 72/28). One electrophoretic analysis of serum was made (Figure 1), and showed albumin (albumin plus alpha 1),[2] 53.9 per cent; alpha globulin, 12.9 per cent; beta globulin, 24.6 per cent; gamma globulin, 8.6 per cent. *Serum cholesterol* was 167 mgm. per cent. *Roentgenograms:* There were numerous small lesions in skull, mandible, ribs, scapulae, and pelvic bones, suggesting multiple myeloma. *Sternal puncture:* Most of the cells obtained were characteristic myeloma cells ("plasma" cell type). The *urine* was very pale and clear. It contained large quantities of a Bence-Jones protein which, on being heated, precipitated at low temperature, disappeared on boiling, reappeared on cooling, and dissolved in strong acetic acid. Many examinations of the urine were made, and usually it became clear on boiling. Occasionally, however, slight cloudiness persisted at the boiling point. It was always evident that nearly all of the protein was soluble at high temperature. No red blood cells, occasional leukocytes, and rare casts were found microscopically.

Course of the disease. Repeated transfusions were given, but the anemia increased, nose-bleeds developed, and there was evidence of progressive renal insufficiency.

When the patient was first examined, the blood NPN was 106 mgm. per cent. During the next 5 months it rose gradually to 156 mgm. per cent, and the urea clearance was found to be 3 and 4 per cent of normal standard. Death occurred 2 months later, about 1 year after the onset of symptoms. Permission to perform an autopsy could not be obtained.

Materials examined and methods used. During the last

[1] Aided by a grant from the Rockefeller Foundation Fluid Research Fund of the School of Medicine of the Johns Hopkins University.

[2] The protein described by Longsworth (4) as alpha 1 globulin has been found by Davis (3) to be even more soluble on fractional salting-out than electrophoretic serum "albumin," and we have therefore included it with the "albumin" fraction of the blood serum.

5 months of the disease, 12 twenty-four hour specimens of urine, varying in volume from 1,200 to 2,050 cc., were collected, and samples of each were analyzed by a chemical method for total protein and for the relative proportion of albumin and globulin. The total protein was precipitated by means of 20 per cent trichloroacetic acid. Globulins were precipitated by Howe's primary and secondary potassium phosphate mixture (5). Nitrogen determinations were done by the macro-Kjeldahl method. The methods used for collection and preservation of urine, and the technique employed in the chemical analyses of urinary protein, have been described in detail (1).

Electrophoretic analysis of blood serum and urine was performed by the modification of the apparatus of Tiselius described by Longsworth and MacInnes (6). The urinary proteins were precipitated by saturation with ammonium sulfate, filtered, redissolved, and dialyzed in running tap water until essentially free of ammonia. They were then dialyzed against a barbital buffer of ionic strength 0.1 and pH 8.5. The electrophoretic measurements were made of the ascending pattern. The mobilities of the various protein fractions were not determined.

Results. The results of the urinalyses are shown in Table I. The total protein in the 12 specimens of urine varied from 0.475 to 0.744 grams per cent. Nearly all of the protein in each specimen was found to be globulin by both the chemical and electrophoretic methods of analysis. The largest amount of albumin in any of the specimens was found 9 days before death when the urine contained 20 per cent albumin and 80 per cent globulin. The single specimen of blood serum which was analysed electrophoretically contained 24.6 per cent of beta globulin, the normal usually being about 15 per cent or less of the total protein (7, 8).

DISCUSSION

There is evidence that Bence-Jones proteins originate from cells of the bone-marrow (9); and it has been found that there are a number of Bence-Jones proteins which may differ from each other in solubility (10), antigenic properties (11, 12), electrophoretic mobility (7, 13 to 15), and molecular size (15 to 17).

It seems evident, and it has been shown to be true, that in cases of multiple myeoloma with Bence-Jones proteinuria, the protein may be demonstrated in the blood serum (14, 15, 18 to 20). Many of the Bence-Jones proteins have been found to salt-out (14, 15, 19, 21, 22) and to behave electrophoretically like globulins (14, 15). In the case described above, the blood serum contained an excess (24.6 per cent) of electrophoretic beta globulin. Most of the urinary protein moved electrophoretically like beta globulin, salted-out like a globulin, and exhibited the solubility characteristics, when heated, boiled, and cooled, typical of a Bence-Jones protein. It seems clear that electrophoretic beta globulin of the serum was excreted in the urine as Bence-Jones protein.

The blood serum of this case, fractionated on two occasions by the Howe sulfate method, appeared to contain an increased proportion of albumin (72 and 74 per cent of the total protein). Electrophoretic fractionation, however, showed only 53.9 per cent albumin. Similar differences in the results of chemical and electrophoretic analyses have been described by others (14, 15). One case of apparent hyperalbuminemia reported was found to be due to the presence in

TABLE I

Chemical and electrophoretic fractionation of urinary protein

	Chemical method (Precipitation of globulins by Howe's phosphate mixture)					Electrophoretic method				
						Relative percentage of albumin and of globulin fractions				
	Protein nitrogen	Albumin nitrogen	Total protein	Relative albumin	Relative globulin	Albumin	Globulin	Alpha globulin	Beta globulin	Gamma globulin
	grams per cent			*per cent*						
September 7, 1941	0.083	0.000	0.519	0	100	0	100	0	98	2
October 1, 1941	0.090	0.000	0.563	0	100	0	100	0	100	0
October 10, 1941	0.119	0.006	0.744	5	95	0			99+	
October 18, 1941	0.104	0.006	0.650	6	94					
October 24, 1941	0.110	0.000	0.688	0	100					
October 31, 1941	0.114	0.000	0.713	0	100					
November 7, 1941	0.104	0.000	0.650	0	100					
November 14, 1941	0.090	0.000	0.563	0	100					
November 30, 1941	0.077	0.000	0.481	0	100					
December 12, 1941	0.091	0.000	0.569	0	100					
January 9, 1942	0.076	0.006	0.475	8	92	7		0	91	2
January 16, 1942	0.076	0.015	0.475	20	80					

the serum of a protein which had the electrophoretic mobility of beta globulin, and was excreted in the urine as Bence-Jones protein. In the urine, it maintained the electrophoretic mobility of beta globulin and salted-out like a globulin.

It is not clear why Bence-Jones protein is excreted in the urine of some cases of myeloma with Bence-Jones proteinemia and not in others. The molecular weights of the Bence-Jones proteins so far determined have been found to be in the neighborhood of 37,000 (15, 16, 23, 24). Proteins of this molecular size might be expected to pass through normal glomerular capillaries, since the Bence-Jones proteins are smaller than serum albumin whose molecular weight is approximately 65,000 (25). There is evidence, however, suggesting that in addition to molecular size, other as yet unrecognized factors are also concerned in determining the presence of Bence-Jones proteinuria. The excretion of serum albumin in many cases, in addition to Bence-Jones protein, seems to require some other explanation; and a case was recently reported in which there was hyperbeta-globulinemia without Bence-Jones protein in the urine, although the molecular size of the abnormal serum beta globulin was consistent with that of other Bence-Jones proteins which were excreted in the urine (14, 15).

In some cases of myeloma, a form of renal insufficiency develops, due chiefly to obstruction of tubules by precipitated Bence-Jones protein (26 to 30). The suggestion has been made that prolonged Bence-Jones proteinuria of large quantity may be necessary for the development of this type of renal insufficiency (29, 31). Such an explanation is supported by the association of marked chronic proteinuria and progressive renal insufficiency in the case described in this paper, and also by recent studies concerning the effect of proteinuria on the kidney (1 to 3). Chemical and electrophoretic analyses of urinary protein have indicated that, in various forms of acute and chronic Bright's disease, the rate at which protein coagula collect in glomeruli and tubules is probably determined chiefly by the presence of high relative proportions of globulin (30 to 35 per cent or more of the total protein) in urine which contains total protein in high concentration (0.5 per cent or more). In cases of lipoid nephrosis, low proportions of globulin in the urine are associated with the absence of appreciable numbers of protein coagula in the kidney and the failure of renal insufficiency to develop (1 to 3, 32).

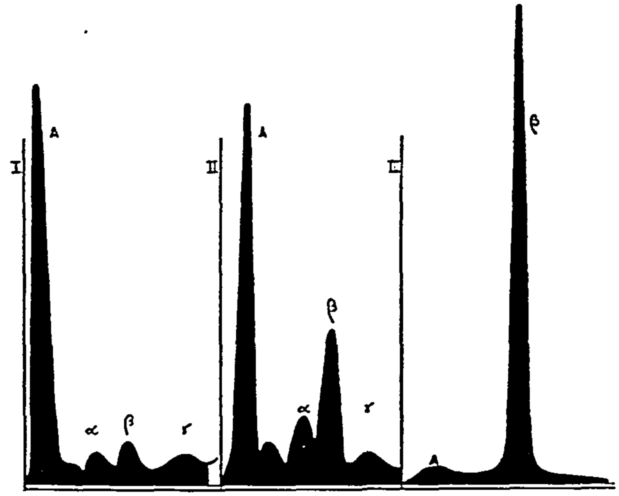

Fig. 1. Electrophoretic Patterns (Ascending Limbs) of Normal Human Blood Serum (I), and of the Serum (II) and Urine (III) of the Patient (M.T.) with Multiple Myeloma

The mechanism responsible for precipitation of Bence-Jones and other serum proteins within the kidney is probably concerned not only with the concentration of the proteins in the urine but also with other factors which remain to be investigated. It is probable, however, that the concentration of salts and urea in the urine are important. Under these conditions, the proteins salting-out as globulins would doubtless precipitate in the kidney, as in the test tube, more readily than the albumins. The analyses of urinary proteins excreted in Bright's disease of various types (1 to 3, 32), including the present case of renal failure with myeloma, indicate that it is chiefly the globulins, rather than albumins, which collect within the glomeruli and obstruct the renal tubules.

SUMMARY

The protein in the urine of a patient with multiple myeoloma and progressive renal insufficiency was shown by the usual solubility methods to be a Bence-Jones protein. The urine was also analyzed electrophoretically and by a chemical method. These two methods gave

very nearly identical results. By both methods of analysis, the Bence-Jones protein behaved like a globulin. Electrophoretically it moved like the beta globulin of normal blood plasma.

The total plasma protein of the patient was normal, but there was a marked increase in the electrophoretic beta globulin fraction, which was excreted in the urine as Bence-Jones protein.

During the period of observation, the concentration of protein in the urine varied from 0.475 to 0.744 grams per cent, and the proportion of Bence-Jones protein usually varied from 92 to 100 per cent of the total. The evidence suggests that in multiple myeloma, as in other forms of Bright's disease, the development of that portion of renal insufficiency which is caused by the precipitation of plasma proteins within the kidney is determined chiefly by the duration of high concentrations in the urine of proteins which have the salting-out and electrophoretic properties of globulins.

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ATYPICAL PNEUMONIA CAUSED BY PSITTACOSIS-LIKE VIRUSES [1]

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Previous reports (1 to 7) have indicated that some of the more severe forms of atypical pneumonia may be caused by any one of a group of viruses related to psittacosis and meningopneumonitis. In several instances (1 to 5, 14), virus has been isolated from the patient. More often, however, the diagnosis has been made on the basis of the complement-fixation test which distinguishes this group of infections from other primary atypical pneumonias, but fails to differentiate between the individual viruses within the group.

This paper will present data on 10 cases of atypical pneumonia due to psittacosis-like viruses in which the etiology was determined by isolation of the virus and by serological tests, and 2 related cases which apparently were sources of infection. The strains of virus isolated fall into 2 types, one of which probably originated in pigeons and is designated pigeon ornithosis virus (2, 8, 10), the other being of unknown origin, but probably identical with the strains isolated in 1940 (1) and designated S-F. A laboratory infection due to the meningopneumonitis virus (9) is also described.

CLINICAL AND EPIDEMIOLOGICAL FINDINGS

Cases due to pigeon ornithosis virus. Four naturally occurring infections with pigeon ornithosis virus were studied. All of these gave a history of contact with pigeons, though in one case this contact was very transient. All 4 cases were severe, presenting the picture of systemic infection with broncopneumonia of variable extent, and 2 terminated fatally. One death was peculiar in that it occurred after the infection had apparently subsided and presumably was due to the age and cardiovascular status of the patient. The other death occurred in a patient with fulminating infection and rapidly extending bronchopneumonia. The clinical, epidemiological, and laboratory data from these cases are summarized in the first part of Table I. The virus of pigeon ornithosis was isolated in our laboratory from the sputums of all 4 patients, and in 2 cases, a similar virus was isolated by Dr. K. F. Meyer from the pigeons in the flocks presumably responsible for infection of the patients.

Case 1 (Figure 1). B. S., male, 63, became ill on May 7, 1942, complaining of sore throat, cough, headache, chills, anorexia, and diarrhea. On May 11, he was admitted to Mt. Zion Hospital. Temperature was 106° F., pulse 120, irregular, respirations 24, blood pressure 132/82. Physical examination showed an elderly male of poor nutrition who was irritable and uncooperative. The pharynx was beefy red. Scattered râles were heard at the right base. The spleen was felt at the costal margin.

Laboratory findings: White blood count was 6,200. Urine contained albumin (+). Chest x-ray showed an area of uniform greyness in the L.U.L. which was interpreted as "virus pneumonia." E.C.G. showed auricular flutter and fibrillation. Routine bacteriological and serological tests were negative. White blood count fell to

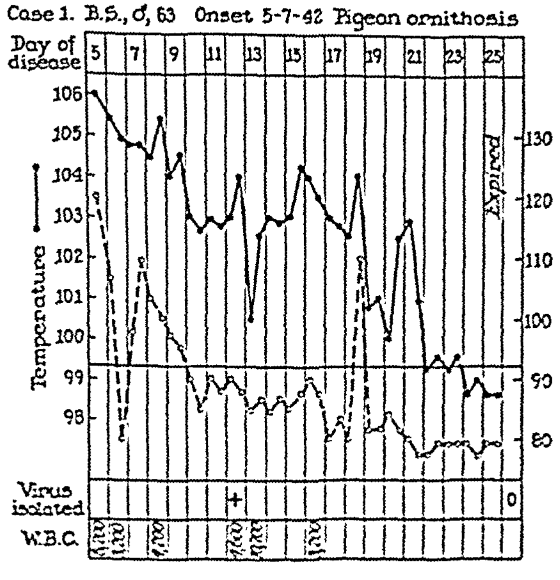

FIG. 1. CASE DUE TO PIGEON ORNITHOSIS VIRUS

[1] The studies and observations on which this paper is based were supported by the International Health Division of The Rockefeller Foundation.

TABLE I

Summary of cases

Case number	Name	Age	Sex	Duration of fever	Height of fever	White blood cells	Virus isolated*		Complement fixation		Epidemiology
							Day of illness	Type	Day of illness	Titer	
		years		*days*	° *F.*						
1	B. S.	63	M	25+	106	3,200 to 6,200	12	P.O.	25	>1 : 32	Pigeon contact. Similar virus recovered from other birds in flock
2	M. P.	38	M	35	105.2	5,600	24	P.O.	31 46	>1 : 256 >1 : 256	Transient pigeon, parrot, and chicken contact. Bird sera negative by C-F test
3	A. B.	36	F	20	104.3	8,450	9	P.O.	9	1 : 32	Fed flock of wild pigeons
4	R. C.	48	M	13+	105.6	7,200 to 10,000	8	P.O.	9	1 : 4+	Pigeon contact. Cleaned loft 2 weeks before onset of illness. Similar virus from pigeons
5	M. C.	24	F	16	104.4	9,000	8	M-P	pre 25	0 1 : 32	Laboratory infection
6	J. P.	54	M	14+	107	6,000 to 7,900					Source of infection undetermined
7	L. S.	53	F	13+	105.8	12,200 to 5,800	13	S-F (lung-spleen)			Nurse. Contact with Case 6
8	N. F.	35	F	13+	105.4	8,850 to 27,560	13	S-F (lung)			Nurse. Contact with Case 6
9	H. P.	29	M	14	105.2	6,300 to 10,500	8	S-F	pre 21	0 1 : 16	Laboratory infection
10	M. B.	42	F	15	103.6	11,000	14	S-F	8 17	1 : 16 1 : 128	Laboratory infection
11	M. M.	35	M	14+	105	6,200					Source of infection undetermined
12	M. D.	53	F	13+	105.8	8,700 to 10,400	13	S-F (lung)	10	1 : 8+	Nurse. Contact with Case 11

+denotes fatal case.

* P.O. = Pigeon ornithosis virus; M-P = meningopneumonitis virus; S-F = pneumonitis virus identical with type strain S-F. Viruses isolated from sputum unless otherwise noted.

3,200 on May 22. X-rays taken on May 14 and May 21 showed an extension of the infiltration in the L.U.L., but no spread to other lobes.

Course: The patient ran a severe febrile course with frequent chills. Temperature ranged from 101° to 104° F. from the 9th to the 21st day. Auricular fibrillation continued, but the ventricular rate was controlled by digitalization. Sputum became copious and was at times rusty in character. He became incontinent on May 15 and irrational on May 19. From that time on he refused to take food. A right-sided parotitis complicated his illness between May 18 and May 24. Despite his cardiac status and poor nutritional state, the patient's temperature gradually fell and reached normal on May 28. Chest findings cleared. He remained irrational and on May 30 became markedly worse and died on May 31, 1942.

Autopsy findings: Postmortem examination was remarkable for the paucity of positive findings. Small amounts of clear fluid were found in both pleural cavities. There was no consolidation in either lung, nor was there evidence of pulmonary embolus. The spleen was enlarged to three times normal size and on section was greyish-red in color, pulpy in character. Microscopic examination of lung section taken from the L.U.L. showed thickening and fibroplastic proliferation in the interstitial tissue, areas of atelectasis, flattening of bronchiolar epithelium, and scattered aggregates of large and small round cells. The serous exudate in the alveoli was entirely devoid of nucleated cells.

Isolation of virus: The virus of pigeon ornithosis was isolated from sputum obtained on the 10th day of disease, but efforts to recover the same agent from lung and spleen tissue obtained at autopsy were unsuccessful.

Epidemiology: Shortly before the onset of his illness, B. S. had obtained 4 pigeons with the intention of eating them. The birds were not alive at the time of investigation and were presumably eaten. Other members of his family did not become ill. Three pigeons of the flock from which B. S.'s pigeons originated were examined by Dr. Meyer at the Hooper Foundation. From the livers and spleens of 2 birds, the virus of pigeon ornithosis was isolated. Dr. Meyer has very kindly allowed us to use this strain in cross-immunity tests to be described later in this paper. It was further learned that the original owner of this flock, J. C., male, 60, had died on April 19, 1941 as the result of an atypical pneumonia. The exposure to presumably infected birds and the nature of his illness offer suggestive, though by no means conclusive, evidence that he also was infected with the virus of pigeon ornithosis.

Case 2. M. P., male, 38, noted malaise, fever, and cough on May 7, 1942 and was admitted to the hospital about one week later. Chest x-ray showed pneumonic infiltration in the right hilar region. For 3 weeks, the patient remained seriously ill, running a high spiking fever. Temperature reached normal on June 11, 1942. Convalescence was slow but uneventful.

Epidemiology: Two weeks prior to the onset of his illness, M. P. had visited a relative who owned a parrot, 8 pigeons, and 8 chickens. His visit lasted one-half hour. He did not handle any of the birds. With his wife, he picked and later ate some lettuce planted close to the pen in which the pigeons were kept. All the birds appeared healthy. All pigeons and chickens were bled and failed to show complement-fixing antibodies to psittacosis virus. According to Meyer, however, this does not exclude latent infection. The evidence that M. P. acquired his infection during this visit is suggestive only.

Case 3. A. B., female, 36, became ill on July 3, 1942, complaining of malaise, fever, and cough with bloody sputum. She was admitted to the hospital on July 5. Physical examination showed an acutely ill patient with an extensive bronchopneumonia. On July 7, marked dyspnea and cyanosis were noted. Dyspnea persisted throughout the serious phase of her illness. Following a week during which the patient's temperature ranged between 102.6° and 104.3° F. and her condition appeared critical, gradual improvement occurred. Temperature remained normal after July 22, 1942.

Epidemiology: For a considerable period, A. B. had fed a flock of wild pigeons from the back window of her apartment, the closeness of contact being undetermined. It was impossible to obtain any of the birds for study.

Case 4. R. C., male, 48, became ill suddenly on March 19, 1943, complaining of generalized aches and pains, sore throat, "terrific" headache, nausea, and vomiting. He was admitted to the hospital on March 23. Temperature was 103.2° F., pulse 88, respirations 22. On physical examination, he appeared acutely ill. Pharynx was extremely red and bronchial breathing was noted over R.M.L. and L.L.L. Severe chills occurred repeatedly. Cough was severe and produced blood-tinged sputum. Abdominal distention was troublesome. Temperature remained between 102° and 105.6° F. Respirations rose steadily and exceeded 30 during the second week of illness. X-ray on March 24 showed an infiltrating lesion in the L.L.L. with accentuated bronchovascular markings throughout. On March 31, the pneumonic infiltration had spread and areas of increased density were observed in the R.U.L., R.M.L., and scattered throughout the left lung field. On March 28, the patient became irrational. Pulse rate, which had remained below 110, rose rapidly. Death occurred on April 2, 1943. Autopsy was not performed.

Isolation of virus: Sputum obtained on March 29, 1943 was inoculated intranasally into 2 cotton rats. Both appeared ill on the second day. One was sacrificed on the 4th day and showed extensive pulmonary infiltration. Impression smears of the lungs contained numerous elementary bodies. Passage in mice showed that the strain had the properties of the pigeon ornithosis virus.

Epidemiology: In December 1942, R. C. acquired 32 pigeons which he kept in a shed behind his house. Two weeks before the onset of his illness, he cleaned the shed for the first time, removing all the dry and dusty droppings. Seven pigeons were obtained and taken to Dr. Meyer for testing. Six of these showed strongly positive complement-fixation tests for psittacosis and virus was recovered from 6 birds. None of the 4 other members of the family, who had only casual contact with the pigeons, showed evidence of infection or significant elevation of complement-fixation titer.

Cases not related to contact with birds. These include 3 cases in nurses and 3 laboratory infections. The data on these 6 cases and 2 related cases are summarized in Table I.

Case 5. M. C., female, 24, a laboratory technician, became ill on March 20, 1942 with chills, fever, malaise, cough, and severe headache. The course was milder than in any of the other cases and there was very little clinical or x-ray evidence of pulmonary involvement. M. C. had been using meningopneumonitis virus grown in chick embryos for complement-fixation tests, but had no known contact with birds. A virus indistinguishable from meningopneumonitis and pigeon ornithosis was isolated from sputum by intranasal inoculation of cotton rats and mice.

The strain of virus designated S–F was originally isolated from 2 nurses who presumably became infected while taking care of a patient with an atypical pneumonia. Brief descriptions of these cases were presented in the original report (1). Clinical data, however, were not presented but are of sufficient interest to warrant a brief summary here.

Another patient-to-nurse infection occurred in 1942 and a virus identical with the original S–F strain was again isolated. In addition, there were 2 laboratory infections

with this strain which differed from the naturally occurring cases only in the fact that they were somewhat less severe.

Case 6. J. P., male, 54, became ill on March 5, 1940 and was admitted to the hospital on March 8. X-ray showed bronchopneumonia at the right base. Blood culture was negative. Fever remained high and no effect was noted following treatment with sulfapyridine. The patient died on March 18, 1940. Autopsy was not performed and no sputum or blood specimens were obtainable.

Case 7 (Figure 2). L. S., female, 53, a general duty nurse on the floor on which J. P. was a patient had numerous opportunities for exposure between March 8 and March 12, 1940. She became ill suddenly on March 25, complaining of severe frontal headache, nausea, dull abdominal pain, and a severe chill which lasted 10 to 15 minutes. On admission to the hospital on March 26, temperature was 101.8° F., pulse 86, respirations 24. Physical examination was entirely negative. White blood count was 12,200 (polymorphonuclear leukocytes 88 per cent). X-ray showed an area of increased density in the upper right lung field which was interpreted as an area of bronchopneumonia. Blood culture was negative. The patient's course was extremely stormy, with temperature for the most part between 103° and 105.8° F., pulse 86 to 110, and respirations, during the second week, consistently over 42. Death occurred on April 7, 1940.

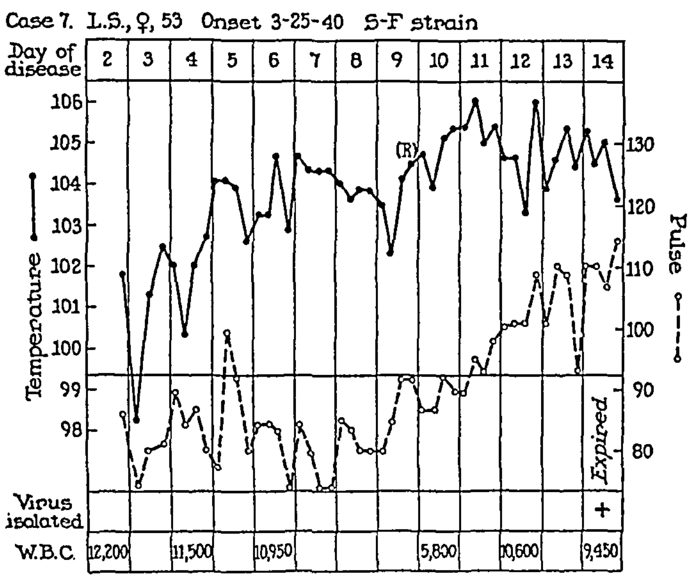

FIG. 2. CASE DUE TO S-F STRAIN, NOT RELATED TO CONTACT WITH BIRDS

Autopsy showed a lesion involving most of the right upper lobe, the tissue being rather solid, but still slightly crepitant and greyish-red in color. A thin fibrinous exudate covered the pleura. Spleen was large and soft. On microscopic examination, the pulmonary alveoli were found to contain a loose exudate of fibrin with only moderate numbers of cells. In most areas, large mononuclear cells predominated with scattered areas of hemorrhage and very few lymphocytes or polymorphonuclear leukocytes. Many macrophages were loaded with pigment probably derived from hemoglobin. Parts of the involved lobe contained a denser, more cellular exudate with relatively larger numbers of polymorphonuclear leukocytes. The pathology in this case was very similar to that of psittacosis (11) and also to certain other virus pneumonias not caused by psittacosis-like agents. A psittacosis-like virus (strain S–F) was isolated from lung and spleen (1) obtained at autopsy.

Case 8. N. F., female, 35, was a relief nurse who had brief exposures to J. P. between March 8 and March 18, 1940. On March 25, she noted severe headache and anorexia. She was admitted to the hospital on March 27 and died on April 7, 1940. Clinical course and postmortem findings were very similar to Case 7 except that on physical examination, stiffness of the neck and Kernig's sign were observed.

Of 3 special nurses who cared for J. P. from March 12 to March 17, 1940, one, B. M., female, 35, became ill with a pneumonia similar to those described, but recovered after a prolonged illness. Clinical information was not obtained and etiological studies were not done. Complement-fixation test with psittacosis antigen was positive in a dilution of 1 : 256 following recovery.

Epidemiology: J. P. died as the result of a pneumonia of undetermined etiology. No history of contact with psittacine birds or with pigeons was obtained. Three of 15 nurses who cared for him acquired similar infections, 2 terminating fatally.

Cases 9 and 10 were laboratory infections which occurred during investigation of the S–F virus.

Case 9. H. P., male, 29, noted weakness on August 10, 1940. On August 11, he became acutely ill with headache, malaise, chill, and fever. He was admitted to the hospital on that day. Temperature was 103.2° F., pulse 94, respirations 24. Physical examination showed a flushed, feverish patient. Pharynx was moderately inflamed. Chest examination was negative.

Course: Temperature remained high, ranging from 102° to 105.2° F. Cough with scanty sputum was noted on August 12. Moist râles were heard at the left base on August 16. On August 19, marked prostration, lethargy, and incontinence were observed. Chest examination showed dullness, tubular breath sounds, and showers of râles at the left base. On August 20, 100 cc. of serum from a patient convalescent from a similar illness (the nurse B. M., connected with Cases 6, 7, and 8) was given intramuscularly. Marked improvement was noted the following day. An additional 50 cc. of convalescent serum was given on August 22. The patient's temperature fell to normal on August 23, 1940, and remained normal thereafter. Recovery was fairly rapid.

X-ray on August 16 failed to show evidence of pneumonia. On August 20, chest x-ray showed a large patch of pneumonia at the left base. On August 23, this area of infiltration had almost entirely cleared.

Case 10. M. B., female, 42, became ill suddenly on August 15, 1940, 4 days after H.P. Her complaints were severe backache, chill, fever, headache, nausea, and vomiting. On August 18, she began to have non-productive

cough and aching pains in the left chest and shoulder. On August 22, she was admitted to the hospital. Temperature was 101° F., pulse 120, respirations 30. Physical examination showed a moderately ill patient with evidence of bronchopneumonia in the L.L.L.

Course: Following 6 stormy days during which temperature rose to 103.6° F., the patient's condition gradually improved. Temperature reached normal on August 29, 1940, and remained normal apart from a spike of fever on September 3. Convalescent serum from a patient who had recovered 5 months previously from a similar illness and from a normal donor with high antibody titer for psittacosis was given intramuscularly, in small daily doses, from August 23 to August 25, 160 cc. in all. No clear-cut effect was noted. Convalescence was extremely slow and was complicated by severe neuritis and arthritic pains.

X-ray on August 22 showed bronchopneumonia in the L.L.L. Later films showed a migratory type of pneumonia, involving in succession the midportion of the left field, the left base, and the right hilar region. By September 3, these areas had cleared for the most part, but new areas of infiltration were noted at the right base.

Case 11. M. M., male, 35, was admitted to the hospital on January 28, 1942, suffering from an extremely severe diffuse bronchopneumonia of approximately one week's duration. His temperature ranged from 103.8° to 105° F. White blood count was 6,200. Sulfonamide drugs were given without benefit. He failed rapidly and died on February 3, 1942. Autopsy was not performed.

Case 12. M. D., female, 53, a nurse, was in contact with Case 11 from January 28 to February 3, 1942. On February 10, she suddenly became ill with high fever, repeated severe chills, headache, anorexia, nausea, vomiting, and cough. Sputum was very scanty. Her condition rapidly became worse and she was admitted to the hospital on February 17. At that time, temperature was 105° F., pulse 84, respirations 24, blood pressure 160/80. On physical examination, the patient appeared seriously ill, though lying quietly flat in bed with only slight dyspnea. Pharynx was injected. Chest examination showed dullness at the left base and bronchial breath sounds and moist râles over the whole left side. Spleen was not felt.

Laboratory findings: Urine showed a heavy trace of albumin. Sputum, obtained by throat swab, showed no pneumococci, but a heavy growth of hemolytic staphylococcus aureus. X-ray on February 17 showed a moderate amount of fluid in the left costophrenic angle, a diffuse patchy bronchopneumonia of the whole left lung, and a small amount of patchy pneumonia at the right base.

Course: Sulfadiazine was given in doses of 6.0 grams daily and a blood level of 14.0 mgm. per cent was obtained without apparent benefit. On February 18, 110 cc. of clear yellow fluid were removed from the left pleural cavity. This fluid was sterile on culture. On February 20, the patient suddenly became cyanotic and unconscious with marked generalized rigidity. She remained in coma until her death on February 22, 1942. Autopsy was not performed.

Isolation of virus: One cc. of fluid was obtained by lung puncture on February 22. Culture showed hemolytic staphylococcus aureus. Virus was obtained by mouse passage.

Epidemiology: Although the etiology of the pneumonia in the original case was not determined, the similarity of his illness and the known person-to-person communicability of this agent makes it likely that M. D. contracted her infection as a result of exposure to him. The origin of M. M.'s infection remains uncertain. It was learned that he had lived for 2 years in close proximity to chickens, but no information relative to other avian contact was obtained.

Among 6 naturally occurring infections presumably due to the psittacosis-like strain S-F, 4 occurred in nurses in attendance on the 2 original patients. This points to a high person-to-person communicability. Five of the 6 cases terminated fatally, an exceedingly high mortality. The 2 laboratory infections occurred during routine investigation of the virus and reemphasize the danger of work with agents in the psittacosis group, particularly when inoculations are done by the intranasal route.

Clinical diagnosis. All available evidence indicates that it is impossible to distinguish clinically the infections due to the agents described from each other or from psittacosis. The question arises whether it is possible to differentiate clinically between pneumonias due to psittacosis-like viruses and the prevalent type of primary atypical pneumonia which is not caused by these agents. On the basis of the present data, this question must be answered in the negative. Certain points, however, may be of definite assistance. In general, the large majority of primary atypical pneumonias are mild, with a comparatively small proportion moderately severe, and an even smaller proportion extremely severe or fatal. The case fatality among several hundred cases which we have observed has not exceeded one per cent. With the psittacosis-like viruses, on the other hand, the majority of infections have been moderately or extremely severe and the case fatality has been high. Consequently, it is with the severely ill patient that the question of differentiation becomes important.

Among the symptoms which have been, in our experience, more common in the psittacosis-like group, the following deserve mention. Chills occur more frequently, usually near the onset, and may occur repeatedly during the first week. Gastrointestinal symptoms are frequently pres-

ent. The patient often appears desperately ill even though the area of pulmonary infiltration is not great and respiratory distress is slight. In the correspondingly severe cases in the other group which we have observed, the pulmonary infiltration has involved most of one lung and frequently part of the other, and respiratory distress is extreme. Marked disturbance of cerebral functions is almost the rule. A final point, useful for the most part in retrospect, is the greater duration and height of the fever.

LABORATORY STUDIES

Ten strains of virus have been isolated from sputum or autopsy specimens obtained from the cases of pneumonia just described. The properties of the strains isolated from Cases 7, 8, 9, and 10 in 1940 have been described in detail in previous publications (1, 22, 23). These strains were compared with 6 additional strains isolated in 1942–43.

Method of isolation of virus from sputum or autopsy specimens. Sputum or tissue was ground thoroughly in a mortar with alundum and diluted with plain infusion broth (pH 7.6) or with 10 per cent horse serum broth to make a 10 to 20 per cent suspension. Coarse material was allowed to settle or was centrifuged out at low speed.

Four white mice were inoculated intranasally under ether anesthesia with 0.05 cc. of suspension. The mice were kept in strict isolation. At the end of 4 to 7 days, they were sacrificed, the lungs removed with sterile precautions, and examined for areas of consolidation, particularly small round grey foci as described by Eaton, Beck, and Pearson (1). Cotton rats were used in some cases for first passages. Pulmonary findings resembled those in mice. Subsequent passages were carried in mice.

The first passage mouse lungs were ground with alundum and broth to make a 10 per cent suspension and inoculated in amounts of 0.05 cc. intranasally, 0.03 cc. intracerebrally, and 0.5 cc. intraperitoneally into 3 groups of mice. Mice dying from intranasal and intracerebral inoculations were examined for elementary bodies by making impression smears of the lungs and brains and staining by the method of Macchiavello.

Usually, those mice receiving intracerebral inoculations with positive material died in 3 to 5 days and the brain smears showed many elementary bodies. Mice inoculated intranasally did not always die on the second passage, but usually showed marked greyish-red consolidation of the lungs and positive smears.

Psittacosis or pigeon ornithosis is carried at least 21 days or longer in the liver and spleen of mice. On the other hand, it has been reported that the S-F strain cannot be recovered after 9 days from the livers and spleens of mice following intraperitoneal inoculation (1, 23). This variation was considered significant and all new strains isolated were given this differential test. The second-passage mice inoculated intraperitoneally were held for 21 days. They were then autopsied and examined particularly for enlargement of liver and spleen, hepatic necrosis, peritoneal exudate, fibrin, and ballooned intestines. The liver and spleen were ground in a mortar and a 10 per cent broth suspension was made. This material was inoculated intranasally and intracerebrally into normal mice to determine if the virus was still present in these organs.

Properties of the strains isolated. All strains included in this study formed minute coccoid elementary bodies, demonstrable in impression smears of the brains and lungs of mice, and all were fatal to mice when inoculated by the intranasal and intracerebral routes. Only 2, however, strain B.S. from Case 1 and strain M.C. from Case 5, showed marked virulence for mice by the intraperitoneal route, B.S. killing irregularly and M.C. killing in dilutions of 10^{-3} and 10^{-4}. Mice inoculated intraperitoneally with each strain were tested at the end of 21 days for the carrier state. Four strains (S-F, H.P., M.B., and M.D.) from Cases 8, 9, 10, and 12, respectively, produced no carrier state at the end of 21 days. However, livers and spleens from mice receiving the strains B.S., M.P., A.B., R.C., or M.C. (Cases 1, 2, 3, 4, and 5) killed mice, subinoculated intracerebrally, in 3 to 5 days and caused lung consolidation with typical elementary bodies at the end of 7 days, in mice subinoculated by the intranasal route.

There was apparently some correlation between the epidemiological history and the properties of the virus in mice. Eliminating the

3 laboratory infections, 3 out of 7 patients gave definite information of exposure to pigeons. In the case of M. P., a history of fleeting exposure to pigeons with negative serology was obtained. The virus isolated from known or presumed pigeon-contact cases (B. S., M. P., A. B., and R. C.) differed in mouse pathogenicity from the strains obtained from 3 secondary cases in nurses L. S., M. F., and M. D.

Cross-immunity tests. The differences between the strains, as demonstrated by pathogenicity after intraperitoneal inoculation of mice, were confirmed by cross-immunity tests. Mice were immunized by 1 or 2 intraperitoneal inoculations with active virus and tested 3 weeks later by the intracerebral route using 10 to 100 minimal lethal doses (MLD) of strain S-F and 10 MLDs of pigeon ornithosis virus. The latter strain was isolated by Dr. Meyer from pigeons connected with Case 1. A summary of the results is presented in Table II. All members of the group

TABLE II

Intracerebral cross-immunity tests with pigeon ornithosis virus and S-F strain against 8 strains from cases of pneumonitis

Case	Strain of virus for immunization	Route	Results after test with strain:					
			S-F			Pigeon ornithosis		
			MLD	D	P	MLD	D	P
1	B. S.	1x IP	10–100	0/6	0/6	10	0/13	0/13
2	A. B.	1x IP	10–100	0/5	0/5	10	0/6	0/6
3	M. P.	1x IP	10–100	0/12	0/12	10	2/11	0/9
5	M. C.	1x IP	10–100	1/13	0/12	10	1/14	0/13
7 and 8	S-F	1x IP	10–100	0/10	0/10	10	6/10	4/4
9	H. P.	1x IP	10–100	0/21	0/21	10	13/14	1/1
		2x IP	10–100	0/7	0/7	100*	6/7	1/1
10	M. B.	2x IP	10–100	0/7	0/7	100*	5/7	2/2
12	M. D.	1x IP	10–100	0/12	0/12	10	14/15	0/1
Controls	nil	—	10–100	22/27	1/5	10	23/23	0/0

1x IP, 2x IP = 1 or 2 immunizing intraperitoneal inoculations.
D = deaths; numerator number of deaths, denominator number of mice tested.
P = residual paralysis/mice surviving.
* = tested with meningopneumonitis virus.

protected completely against the S-F type strain, but strains S-F, H.P., M.B., and M.D. gave no immunity against pigeon ornithosis, the surviving mice in most instances being paralyzed. The remaining strains tested—B.S., M.P., A.B., and M.C.—produced solid immunity to pigeon ornithosis.

The results of cross-immunity tests place the S-F, H.P., M.B., and M.D. strains in the same antigenic group, and distinguish them from the pigeon ornithosis strains B.S., A.B., and B.C. Strains of the S-F type may be distinguished from psittacosis by similar methods (23). Strain M.C. is similar in pathogenicity to pigeon ornithosis and cross immunity is complete to this virus. It would appear to be necessary to correlate pathogenicity in animals, cross-immunity tests, and epidemiological history before a strain of psittacosis-like virus can be finally identified.

DISCUSSION

In the course of the investigations of the group of diseases which is at present called primary atypical pneumonia, it has become clear that a small proportion of these infections is caused by psittacosis-like viruses. This group includes a number of viruses, 3 of which, namely psittacosis, the pigeon ornithosis virus, and the S-F strain, have to date been isolated from naturally occurring human infections. Evidence that these agents may be differentiated by laboratory procedures has been presented (21, 22, 23). Psittacosis has long been known to cause this type of pneumonia and its clinical picture and source of infection have been intensively studied. Recognition of the fact that a related virus, carried by pigeons (10), may cause illness in humans is a recent development and is due almost exclusively to the work of K. F. Meyer and B. Eddie. Their reports (2, 4, 8) emphasize the widespread distribution of this virus in pigeons and also point out that it may be found in doves and chickens (3). Other reports indicate that this agent is present on other continents (16, 17).

The source of viruses represented by the type strain S-F, whether avian or non-avian, is still undetermined. From the available data, it appears that strains of this type have a relatively higher virulence for human beings and a lower virulence for birds (1, 23) than the pigeon ornithosis virus. Person-to-person communicability, such as from patient to nurse in Cases 7, 8, and 12, is of importance because the secondary infections are frequently severe or fatal. Strains of the S-F type may represent viruses which have become adapted to human beings as a result of one or more direct human transmissions, losing

in the process some of the pathogenicity for mice and birds and part of the antigenic composition of classical psittacosis strains. As a provisional term, *pseudo-psittacosis* is suggested to designate those atypical pneumonias caused by strains like the S-F type which are of undetermined origin and which differ significantly in properties from the viruses of psittacosis and ornithosis. When the origin of the S-F type strains is definitely determined, a name based on the source could replace this group nomenclature.

Other psittacosis-like viruses have been isolated from animals. Although the meningopneumonitis virus was isolated by Francis and Magill from ferrets (9), the close similarity of this agent to ornithosis virus suggests an original avian source. Recently, viruses differing in several properties from those described above have been isolated from mice (18) and cats (19). To date, none of these animal viruses has been recovered from human cases, a possible exception being the laboratory infection with menigopneumonitis virus (Case 5).

The frequency of infections due to the psittacosis-like viruses in this area is not great when compared with that of the primary atypical pneumonias as a whole. During the period of this study, over 250 specimens of sputum or lung from patients with primary atypical pneumonia have been inoculated intranasally into mice, cotton rats, or both. Either animal is very susceptible by this route. Psittacosis-like viruses have been recovered only 10 times. Data acquired by complement-fixation tests suggest that four or five other cases of psittacosis-like infection from which no virus was isolated also occurred. Small antibody increases have been observed in various acute respiratory diseases for which the causative agent could not be identified. It remains to be determined whether these are due to a minor antigenic component in other etiologic agents, to anamnestic reactions, or to heterogenetic immunity. In several cases, an unrelated agent was apparently transmitted to cotton rats (25).

Certain previous reports (6, 14, 20) have put the incidence of psittacosis-like infection much higher than in our experience. The explanation for this difference probably lies in the selection of material. When the incidence of primary atypical pneumonias has been low, psittacosis-like viruses may be responsible for a relatively large proportion of the cases. When primary atypical pneumonia has occurred in epidemic form, as reported by Dingle and his co-workers (15), the proportion of cases due to psittacosis-like infection has been low.

Although the number of infections due to the psittacosis-like viruses is not large, these cases are important because of their severity and high mortality. Mild infections with the psittacosis-like viruses undoubtedly occur, but they have been uncommon in the group which we have studied. On the other hand, primary atypical pneumonias caused by other agents (25) are usually not severe. The number of deaths caused by psittacosis-like agents approaches in frequency the number from all other forms of primary atypical pneumonia.

In the majority of recent reports, the diagnosis of psittacosis-like infection has been based on the complement-fixation test. This test as a diagnostic procedure for psittacosis-like infection has many limitations. Because of the group-specific character of the reaction (24) it gives little information as to the type of virus or source of infection. Other diseases, especially lymphogranuloma venereum (21), may produce high antibody titers with psittacosis antigen. Demonstration of complement-fixing antibodies in a single specimen of serum from a case of atypical pneumonia does not by itself justify a diagnosis of psittacosis or a related disease, although the absence of antibodies during convalescence may exclude these diseases. It is possible that, because of the widespread distribution of psittacosis-like viruses in birds and animals, certain normal human beings may have developed antibodies as a result of contact with these agents. It must be remembered that pneumonia of any kind may occur in a person with pre-existing psittacosis antibodies. Even the demonstration of virus or antibodies in birds associated with the case may be coincidental. Isolation of virus from the patient and demonstration, whenever possible, of a definite antibody response associated with the illness constitute, in our opinion, the only satisfactory positive evidence of specific infection.

SUMMARY

1. Clinical and epidemiological data have been presented on 10 cases of severe or fatal atypical pneumonia from which psittacosis-like viruses were isolated. The pigeon ornithosis virus was obtained from 4 cases, a different but related virus (S-F strain) from 5 cases, and the meningopneumonitis virus from one.

2. Laboratory data have been presented which show that the S-F strain can be differentiated from the pigeon ornithosis virus. All cases from which ornithosis strains were isolated gave histories of pigeon contact. No case of infection with the S-F strain gave a similar history, but 3 of these cases were in nurses who were exposed to patients with similar illness.

3. The place of the psittacosis-like infections in the larger group of primary atypical pneumonias has been discussed.

4. The usefulness and limitations of the complement-fixation test in the diagnosis of infections due to psittacosis-like viruses have been reviewed.

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THE DETERIORATION OF COMPLEMENT ACTIVITY IN NORMAL HUMAN SERUM [1]

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In a previous paper (1), normal standards of hemolytic complement activity of normal human serum were established by an accurate quantitative technique (2) and striking decreases were reported occurring during pneumococcus pneumonia, during serum sickness, and after intravenous type-specific antipneumococcus serum, but not after intravenous sulfonamide administration. The complement titrations were, in the majority of cases, performed within 48 hours of the time that the blood was collected, although a few specimens were titrated as long as 72 hours after collection.

Probably because of its importance in many laboratory tests, the deterioration of complement activity of guinea pig serum has been more intensively studied than has that of humans. Nattan-Larrier and Grimard (3) studied the rate of deterioration of guinea pig complement activity at 5°C., using 100 per cent hemolysis as the end-point. They found that the complement activity disappeared in 25 per cent of the specimens kept from 9 to 15 days. Pierret and Breton (4) found that guinea pig serum kept on ice lost 60 per cent of its complement-fixation power in 8 days and 90 per cent in 15 days. Norton, Barfield, and Falk (5), using guinea pig, rabbit, and swine serums, found that complement activity according to Kolmer's technique deteriorated rapidly at 37°C., activity disappearing within 3 days; while at 4 to 6°C., the original activity was retained for 48 hours, and in 7 days, the unit was 2 to 3 times as much as that of fresh serum. Bigger (6), using guinea pig serum, stated that 75 per cent loss of complement activity occurs in 87 hours at 20°C., whereas an equal loss is not noted before 165 hours at 9°C. The method used was Dreyer's modification of Jorgenson and Madsen's technique, where one unit of complement activity is that amount which will cause 60 per cent hemolysis of a sensitized sheep cell suspension. Williamson (7) stated that serum centrifuged to perfect clearness loses one-third of its complement activity in 5 days at ice-box temperature. It is evident that different techniques, at varying temperatures, make it impossible to compare these results.

A similar situation exists, with fewer available data, concerning the rate of deterioration of complement activity in human serum. Nattan-Larrier and Grimard (3) showed that the complement activity of human serum at 5°C. disappeared, in 70 per cent of the cases, after 9 to 15 days. They further observed that complement activity was absent in 41 per cent of human sera kept for 4 days. Bergenhem (8) cites Massol and Grysez to the effect that human serum loses its complement activity after one-half hour at 37°C., while it is characteristic of complement activity that at 20°C. or under, a relatively long time is necessary until it completely disappears.

In the light of these varying reports, it seemed desirable to study further the normal limits and the progressive deterioration of hemolytic complement activity in specimens of human blood serum, by an accurate quantitative technique (2), at temperatures to which blood specimens are commonly exposed, *i.e.*, refrigerator temperature (6°C.) and room temperature (23° to 25°C.).

MATERIALS AND METHODS

Complement activity was estimated in the blood serum of 71 normal individuals of both sexes. Blood specimens were collected by venepuncture and allowed to clot, centrifuged, and the serum pipetted into sterile, dry tubes which were tightly corked when not in use.

Titrations were determined by the technique of Wadsworth, Maltaner, and Maltaner (2). Briefly, the main points in this technique are as follows: A series of tubes containing sensitized sheep red cells, plus varying dilutions of the serum to be tested, are placed for 15 minutes in a

[1] This study was aided in part by a grant from the John and Mary R. Markle Foundation.

water bath, kept at 37° C. The amount of hemolysis in each tube is determined by comparison with carefully prepared standards and then plotted on a logarithmic scale. A straight line can then be drawn through these points to determine the hemolytic complement activity. The end-point in this standardized system is that amount of serum which is required to produce 50 per cent hemolysis. The advantages of this method over those dependent upon the choice of the one tube in which hemolysis begins or ends have been reported by Wadsworth, Maltaner, and Maltaner (2).

Complement activity is reported in this study in terms of the volume of blood serum in milliliters required to produce 50 per cent hemolysis. Since the volume required is inversely related to the complement activity, the larger the number of milliliters indicated, the lower the complement activity in the specimen of blood serum, and vice versa..

NORMAL COMPLEMENT ACTIVITY

The complement activity in the blood serum of 71 apparently healthy individuals has statistically a "normal" distribution as shown in Table I.

No specimen had an index of activity lower than 0.0066 ml. or greater than 0.0028 ml. The great majority of the serums had a complement activity between 0.0040 and 0.0059 ml. The median was 0.0049 ml.

Specimens of blood serum of 62 persons, whose ages were known, were grouped according to age, as in Table II. The ages ranged from 10 to 84 years and the differences in the medians for each group are not significant. Table III shows the grouping of the indices of complement activity of specimens of blood serum of the same 62 persons according to sex and no significant differences were noted. The findings with respect to age and sex are consistent with those reported

TABLE I

Distribution of complement activity of the serum of normal individuals*

Volume of serum*	Normal persons	Median
ml.		*ml.*
0.0020 to 0.0029	1	
0.0030 to 0.0039	7	
0.0040 to 0.0049	28	
0.0050 to 0.0059	27	
0.0060 to 0.0070	8	
Total	71	0.0049

* Complement activity is inversely related to the volume of serum required to produce 50 per cent hemolysis in a standardized system. Therefore, an increase in volume indicates a decrease in complement activity, and a decrease in volume indicates an increase in complement activity.

TABLE II

Complement activity of the serum of normal individuals grouped according to age*

Age	Number of cases	Volume of serum* Range	Median
years		*ml.*	
10 to 29	30	0.0033 to 0.0063	0.0049
30 to 49	22	0.0037 to 0.0065	0.0045
Over 50	10	0.0044 to 0.0058	0.0052

* See note under Table I.

by Meisel and Wasilkowska-Krukowska (9), where the unit used was the smallest amount of blood serum causing the first trace of hemolysis.

Specimens from 41 cases, the total for which the information was available, were grouped according to the number of days between the collection of the specimen and the test for complement activity. Between the time of collection and the time of testing, the specimens were refrigerated at 6°C. Seventeen specimens were tested on the same day they were collected; 18 specimens, 1 day after; and 6 specimens, 2 days after collection. The variations in the median complement activity (Table IV) of these groups of blood serums, kept at 6°C., tested at different intervals following collection, are not significant. Moreover, these changes were insignificant when compared to the relatively large decreases in complement activity occurring during pneumonia, during serum sickness, and when type-specific antipneumococcus horse and rabbit serums were administered intravenously to pneumonia patients (1).

DETERIORATION OF COMPLEMENT ACTIVITY AT REFRIGERATOR AND ROOM TEMPERATURE

The blood serum of each of 17 normal individuals was divided into 2 portions, one of which was kept at 6°C. and the other at 23 to 25°C. Tests were done at intervals until the complement activity was well below the normal range (over 0.0066 ml.). The general trends of the progressive deterioration of complement activity are charted in Table V, where, for the sake of clarity and brevity, only the median values are shown. The median complement activity of blood serum stored at 6°C. is reduced below the

lower limit of normal within 11 to 13 days; whereas at 23 to 25°C., it requires only 2 days to be reduced to the same extent.

Table V also shows the median percentage decrease in complement activity after storage at room and refrigerator temperature. The decrease in activity progresses with time. Thus, after 2 days at room temperature, median complement activity is reduced 46 per cent, while at refrigerator temperature, it is reduced 44 per cent in the interval of 11 to 13 days; and on the fourth day at room temperature, the median complement activity is reduced more than 117 per cent whereas 14 to 17 days at refrigerator temperature are required for similar reduction in activity.

TABLE III

Complement activity of the serum of normal individuals grouped according to sex*

Sex	Number of cases	Volume of serum*	
		Range	Median
		ml.	
Males	46	0.0033 to 0.0065	0.0050
Females	16	0.0039 to 0.0060	0.0047

* See note under Table I.

Complement activity of individual specimens of blood serum decreased to below the normal range (over 0.0066 ml.) at refrigerator temperature (6°C.) on the following days: 2 on the second, 6 on the seventh, 3 on the 11th to 13th, and 6 on the 14th to 17th. At room temperature (23 to 25°C.), the complement activity dropped to similar levels on the following days: 1 on the first, 5 on the second, 2 on the third, 3 on the fourth, 4 on the fifth, and 1 on the seventh day. It is to be noted that the lowest level of the normal range still represents a significant quantity of complement activity. If an arbitrary point such as 0.01 cc. is selected, where most of the complement activity has disappeared, the changes in individual specimens fall into a somewhat different pattern. At refrigerator temperature (6°C.), complement activity reached this arbitrary level on the following number of days: 1 on the third, 3 on the sixth to seventh, 3 on the 8th to 10th, 2 on the 11th to 13th, and 8 on the 14th to 17th. At room temperature (23 to 25°C.), complement activity reached the same arbitrary level on the following number of days: 2 on the third, 4 on the fourth, 8 on the fifth, 2 on the sixth to seventh, and 1 on the eighth.

TABLE IV

Complement activity of the serum of normal individuals grouped according to the number of days between collection of the specimen and testing*

Days	Number of cases	Volume of serum*	
		Range	Median
		ml.	
0	17	0.0039 to 0.0061	0.0049
1	18	0.0036 to 0.0066	0.0045
2	6	0.0028 to 0.0065	0.0052

* See note under Table I.

TABLE V

Complement activity of blood serum of 17 normal individuals according to the number of days of storage and its rate of deterioration at refrigerator (6° C.) and room temperatures (23 to 25° C.)*

Days of storage	Median volume of serum*		Increase in median volume*	
	6° C.	23 to 25° C.	6° C.	23 to 25° C.
	ml.		*per cent*	
0	0.0046	0.0046		
1	0.0050	0.0052	8.7	13.0
2		0.0067		45.7
3	0.0053	0.0081	15.2	76.1
4		0.01		>117.4
5	0.0054		17.4	
6 to 7	0.0064		39.1	
8 to 10	0.0064		39.1	
11 to 13	0.0066		43.5	
14 to 17	0.01		>117.4	

* See note under Table I.

CONCLUSIONS

1. *In* normal individuals, the median amount of human blood serum required to produce 50 per cent hemolysis of a standardized sheep red cell suspension is 0.0049 ml.

2. The median is not significantly changed by age, sex, or a 48-hour delay, at 6°C., between the time of collecting and of testing.

3. At refrigerator temperature (6°C.), the median complement activity of specimens of human serum is reduced beyond the lower limits of normal in 11 to 13 days. The median percentage decrease in this time interval is 44 per

cent. At room temperature (23 to 25°C.), there is a similar reduction in 2 days.

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INFLUENCE OF ANESTHESIA (ETHER, CYCLOPROPANE, SODIUM EVIPAL) ON THE CIRCULATION UNDER NORMAL AND SHOCK CONDITIONS[1]

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In the presence of an injury, anesthesia can aggravate an already existing condition of shock, or if shock is impending, anesthesia can precipitate it. In an attempt to evaluate the importance of several of the circulatory factors involved, we have studied dogs in good condition and in shock (hemorrhage). Particular attention has been given to the influence of varying depths of anesthesia on heart rate, on systolic, diastolic, and mean arterial blood pressure, on central venous pressure, and on blood flow in three vascular beds, femoral, carotid, and mesenteric.

MATERIALS AND METHODS

Animals. Satisfactory data were obtained from a total of 53 mongrel dogs, weighing about 14 kgm. each. Adequate blood flow data were obtained from the following experimental material: 16 dogs received evipal with 17 runs (*i.e.*, change in anesthesia depth from very light to very deep) on 11 dogs in good condition, and 9 runs on 9 dogs in shock; 9 dogs received ether, with 14 runs on 6 dogs in good condition, and 14 runs on 7 dogs in shock; and 11 dogs received cyclopropane, with 24 runs on 10 dogs in good condition and 18 runs on 10 dogs in shock. Occasionally, because of technical difficulties, flow data in a given experiment were not satisfactory and yet the pressure data were good. Accordingly, the pressure data are based upon a larger series than the flow data: evipal pressure data are based upon experiments on 21 dogs; ether, on 21 dogs; and cyclopropane, on 11 dogs.

The animals were heparinized with Connaught Laboratories' heparin before cannulation, and a constant intravenous drip of heparin was maintained throughout the remainder of the experiment.

Anesthesia. Sodium evipal was administered intravenously in 10 per cent solution. Ether was administered initially by open cone induction. The neck was dissected, a tracheal cannula inserted, and ether during the rest of the experiment was administered by means of a closed system (Foregger) with carbon dioxide absorption. Dead space was maintained near normal. Cyclopropane was given initially in oxygen through a cone equipped with a rubber diaphragm which fitted snugly around the dog's snout. After induction, cyclopropane anesthesia was maintained as with ether.

After the recording camera was started, the usual procedure was to allow the animal to become lightly anesthetized, as judged by the character of the respiration and the sensitivity of the corneal reflex. As a further index of depth of anesthesia, in some of the experiments, we used the reflex contraction of the semitendinosus muscle in response to electrodal stimulation of the sciatic nerve. The activity of this reflex was a useful guide to anesthesia depth in the animals in good condition, but not a dependable one in the animals in shock. Starting from a state of light anesthesia, the anesthesia was deepened. In the case of evipal, this was accomplished by additional intravenous injection, deepening the anesthesia quickly. The volatile anesthetics were employed so as to produce a condition of deep anesthesia during a period of about 10 to 15 minutes (Figures 4, 5, 7, 8). Administration was terminated when the animal was near the point of respiratory failure. The dog was then allowed to blow off the agent, after which the procedure was repeated.

The standard preparation involved the dissection of the trachea, both carotid arteries, one external jugular vein (sometimes both), the superior mesenteric or portal veins, and one femoral artery. Dissection was done with electrocautery, with meticulous hemostatis in order to reduce blood loss after heparinization. The preliminary induction of anesthesia and the dissection usually consumed about one hour and a half. After dissection, the animal was heparinized and flowmeter cannulae inserted into the femoral and carotid arteries and superior mesenteric vein. As a rule, the flowmeter cannulation required that the blood flow through the vessel in question be interrupted for a period of 1 to 3 minutes. Cannulae were also inserted in one carotid artery and in the jugular vein and connected with the pressure manometers for recording. The heater currents and counter currents (see Appendix) were then set for each of the 3 flowmeters, and the recording camera started. These procedures consumed an additional hour; thus about 2½ hours elapsed from the time of anesthesia induction until recording was started. The preparation of the animals was necessarily time consuming and involved considerable dissection and trauma, yet at the end of this,

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

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our subjects were as a rule in excellent condition with a mean arterial pressure of 120 mm. Hg or better when the recording began, and characteristically remained so until routine bleeding was started.

Pressures. Arterial pressure was recorded from the cannulated carotid artery. Central venous pressure was measured through a flexible plastic tube inserted in the right external jugular vein and passed down so that its open tip lay near the entrance of the superior vena cava into the right atrium. Zero pressure base-lines for arterial and venous pressures were taken from the same level by opening the chest of the dog after death, severing the thoracic aorta and vena cava, filling the chest with water up to the level of the entrance of the venae cavae into the atrium, and balancing the mercury columns against this level, a modification of a method recommended by Green (1).

Both arterial and venous pressures were recorded optically by means of glass membrane manometers similar to those described by Green (2). The natural period frequency of the arterial systems (approximately 150 per second) was such as to give a satisfactorily accurate recording of peak systolic and diastolic pressures. A plastic tubing, "Saran" (Dow Chemical Company), was used for the hydraulic connections between manometer and cannula. This tubing was essentially non-distensible, sufficiently flexible, and did not crystallize and break after repeated bending.

In order to record at will either full pulse pressures or integrated mean pressures, a hydraulic damping system was installed (Figure 1). The single lead from stopcock A was connected to the cannula by Saran tubing. When full pulse pressures were to be recorded, stopcock A was turned to transmit the pressure wave through the upper of the 2 parallel arms directly to the glass manometer E, and stopcock B was turned off. To obtain records of damped pressure, stopcock A was turned to transmit the pressure wave through the lower of the 2 arms, in which a plug of glass wool (F) had been inserted. At the same time, stopcock B was opened in order to connect an airbell above the stopcock to the system. Stopcock D was kept closed at all times, except when the amount of air in the airbell was to be adjusted for purposes of varying the time characteristics of the damped record. Stopcock C was used solely to connect the manometer to a pressure bottle and mercury manometer for purposes of calibration. The entire system was kept filled with fluid except for the air bubble which was confined above stopcock B. These damped pressures did not, of course, represent an average of systolic and diastolic pressures, but rather an integrated mean equivalent to half the area under the pressure curve during a time constant which could be varied with the size of the airbell. The advantages of this apparatus over a well-damped mercury manometer lie in ready adjustability of the degree of damping, and easy adaptability to the recording of full pulse wave forms or mean pressures from the same manometer. It works equally well for arterial or central venous pressure. Typical tracings of full pressure and integrated mean (damped) pressures, with conversions from one to the other, are shown in Figure 3.

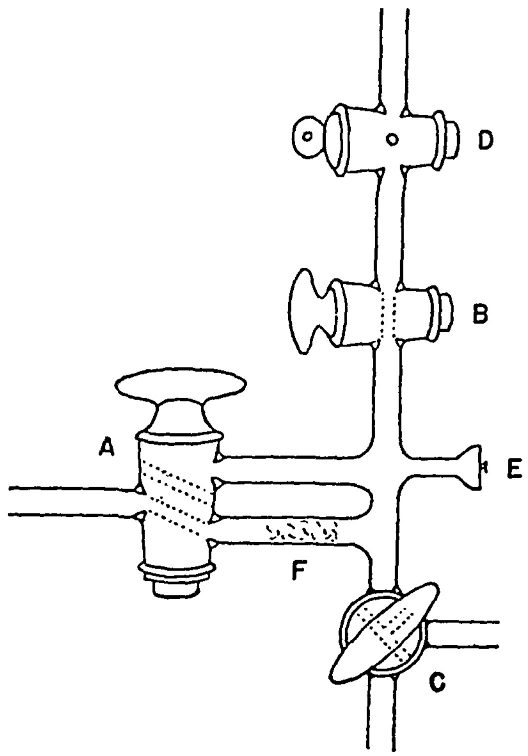


FIG. 1. DIAGRAM OF HYDRAULIC SYSTEM FOR OBTAINING EITHER FULL PULSE PRESSURE TRACINGS OR INTEGRATED MEAN PRESSURES

For explanation see text.

Heart rates and pressures were counted or measured from the optical records. We agree with Werle, Cosby, and Wiggers (3) that because of arrhythmias and variations of systolic and diastolic pressures with individual heart beats, no absolute values for these figures can be taken. In order to minimize the arrhythmic effects of respiration, heart rates were counted for periods which included several respiratory cycles, 10 seconds usually being sufficient. Mean systolic and diastolic figures were estimated from the numerous peaks occurring in a similar period. Mean arterial and venous pressures were measured accurately.

Blood flow. This was recorded optically by means of the heated thermocouple flowmeter of Bennett, Sweet, and Bassett, described in an appendix to this paper. The instrument was improved and refinements added from time to time during the course of the study. Hence, flow records taken early in the study (evipal) are not as reliable quantitatively as those obtained with ether and cyclopropane; but directional changes in the case of evipal are valid as presented. In interpreting the data, due attention was paid to the limitations and inaccuracies of the flow recording method as discussed by Bennett, Sweet, and Bassett. Frequent checks were made for changes in parasitic currents, and any changes found were corrected. The points at which these checks were made show as small breaks in the flow records (Figures 6, 9). When routine corrections were made, a shift appeared in the restored line after the break.

As shown below, there was considerable variation from dog to dog in absolute flow values under comparable con-

ditions when recorded by this method. The majority of the values scattered closely around the mean, but occasional flows far above or below this figure were recorded. Technical errors may be involved in these aberrant values, but changes in their flow on deepening anesthesia showed percentage changes closely in line with those nearer to the mean. Hence, it appeared justifiable to include these flow records in our series, since the results did not conflict with those taken with absolute flow values near to the mean. Possible technical errors in flow recording throw some doubt on the validity of the absolute values for flow, as described in the appendix; but the means cited are not in great disagreement with values for comparable vessels obtained by other methods (4 to 8).

We should like to preface our presentation of the flow data with the clear statement that we realize fully the hazards of blood flow measurement. However, the results are sufficiently consistent to give some basis for conviction as to validity, and pending the development of better methods for recording blood flow, it appears to be justifiable to present the results tentatively, with the hope that they can be checked subsequently when blood flow can be measured with more accuracy.

Impedance calculations. Impedance calculations were made to determine whether or not given flow changes were those to be expected from changes in pressure. An increase in impedance ordinarily indicates vasoconstriction, while a decrease in impedance would denote vasodilatation. The impedance figures are subject to the same errors as those for flow.

From the measurements of flow in a given vascular bed and the difference between the mean arterial and venous pressures, the hydraulic impedance of the bed was calculated by the formula,

$$\text{Impedance} = \frac{\text{Mean Arterial Pressure (mm. Hg)} - \text{Mean Central Venous Pressure (mm. Hg)}}{\text{Mean Flow in cc. per second}} \quad K$$

This calculation is derived from Poiseuille's equation and is similar to the formula commonly used for calculating "peripheral resistance," but differs from the usual formula in that central venous pressure is taken into account, and the word "impedance" is used instead of resistance. Consideration of venous pressure yields a figure of increased accuracy, since, as Poiseuille showed, it is actually the "difference" in hydraulic pressure which provides the moving force to fluid, and in the case of the peripheral circulation, this difference is the difference between the mean central arterial and mean central venous pressures. Both pressures must necessarily be expressed in the same units and with reference to the same level. In dogs, the venous pressure factor makes for only a small error when the animals are in good condition, but in shock, when the mean arterial pressure is low, the error arising from neglect of the venous pressure factor may be as high as 10 to 15 per cent.

The term "impedance" seemed preferable to "resistance" because the animal circulation, with its fluctuating pressures and elastic vessels, bears a close analogy to an A.C. circuit, where inductances and capacitances may produce a lag between voltage and current fluctuations. The term "resistance" which implies a certain special relationship between pressure and flow, properly applies only to hydraulic systems with perfectly rigid walls or with steady pressure head, or both.

Shock production. After one or more "control" runs from light anesthesia to deep and back again, with the animal in good condition, the animal was bled from an artery or from a vein, small amounts at a time, depending upon the condition of the animal. Initially, this usually varied from one-half to one per cent of the body weight with subsequent bleeding at 20 to 30-minute intervals in one-quarter per cent of body weight quantity, depending upon the animal's condition. Bleeding was varied so that over a period of an hour or more the blood pressure would reach a shock level, which was arbitrarily defined as a mean arterial pressure of 70 mm Hg, or below. There was always oozing from cut surfaces after heparinization, and at times, the blood lost in this way was considerable, amounting to over 100 cc. during an experiment of several hours. Blood loss through oozing was as far as possible collected and measured, and included in the figures for total blood loss. Undoubtedly, the dissection and oozing contributed to the shock induced by bleeding. Hence, the shock here is not pure hemorrhagic shock, but is complicated by some trauma from dissection and the necessary intestinal exposure and manipulation and in part by burn from the cautery. When the animal had been put into shock the anesthesia was deepened through one or more cycles as before. This was continued until the animal died.

Records of pressures and of flows were taken continuously from the cannulated vessels. The flow recording beams were checked about every 3 minutes for drift due to variables mentioned before, and any drift found was corrected. The arterial pressure recording system was shifted from damped to undamped at frequent (about 3-minute) intervals during the experiment, allowing us to follow closely changes in systolic, diastolic, and mean pressures. The central venous pressure was usually recorded as damped.

RESULTS AND DISCUSSION

The tables below summarize the data on which the curves (Figures 2, 4, 5, 7, 8) were based. Flow and impedance factors charted and included are expressed in terms of percentage of flow or impedance under conditions of light anesthesia. This afforded the best means of comparing these factors from animal to animal. The averages were computed by taking the flows and impedances during each run at each point in terms of percentage of control flow or impedance value under light anesthesia, and averaging the percentages so obtained. Standard errors of the means are included. Pressure (mm. Hg) and

heart rate data were averaged in terms of absolute values. The points along the time scales during deepening of anesthesia were taken according to the fraction of time elapsed between the point of administering the anesthetic and the time of its termination. During recovery, readings were taken on a straight time basis.

The figures for absolute flow showed considerable variation from dog to dog under comparable conditions, perhaps due in part to variations in body size of dogs and in variations of relative size of head or other members with the various breeds and mixtures that were used. We have corrected our flows and impedances for body surface, believing that individual anatomical differences would be balanced amongst the several dogs in our series.

Flow in cc. per second in femoral and carotid arteries and mesenteric veins was corrected by dividing the flow by body surface in square meters and the corresponding impedances corrected by multiplying by body surface. Body surface was calculated from the body weight by the Meeh formula.

$$\text{Body Surface in Meters}^2 = 0.112 \times \text{Kilograms Body Weight}^{2/3}$$

Table VII shows comparisons of flows in cc. per second per sq. meter of body surface and impedances × sq. meters of body surface, the impedances calculated as in the formula shown above, with the constant taken as 1. The figures are averages of corrected flows and impedances from all our dogs under ether or cyclopropane, the values corresponding to those in the control period of light anesthesia before deepening (Figures 4, 5, 7, 8; Tables III, IV, V, VI).

It will be observed that the mean flow and impedance values shown in Table VII have large standard errors. These large standard errors raise the possibility that technical errors in flow recording may be operative in some cases. As explained by Bennett, Sweet, and Bassett (in the Appendix), opportunity for technical error in the flowmeter used is considerable. The values referred to indicate that this flowmeter has detected no significant differences between femoral, carotid, and mesenteric flows and impedances in dogs in good condition under light cyclopropane anesthesia, as compared with flows in the same vessels under light ether anesthesia when one small group of animals is compared with another group. A comparison of the two agents in a given animal might still show characteristic differences. It would be desirable to repeat the measurements when more accurate methods of recording blood flow become available, as it would also be desirable to study the effects of two agents in the same animal. The percentage changes of blood flow and impedance are more consistent and probably more useful than the absolute values shown.

Sodium evipal

Evipal was the first of the agents studied in the course of these experiments, when the flow recording technic was less accurate quantitatively than later. However, directional changes in flow after evipal injection were consistent, and

TABLE I
Sodium evipal—dog in good condition
Means with standard errors

	Minutes after injection	Arterial blood pressure			Venous pressure	Flows in percentages of control level			Heart rate
		Systolic	Diastolic	Mean		Femoral artery	Carotid artery	Superior mesenteric or portal vein	
			mm. Hg		*mm. Hg*				*per minute*
Control—before injection		133±5	96±5	105±4	−3.3±0.7	100	100	100	171±14
Sodium evipal—10 per cent solution 1.4±0.1 cc. i.v.									
Maximum effect	*1.0±0.2*	*90±9*	*52±6*	*69±10*	*−2.0±0.8*	*43±13*	*53±11*	*61±6*	*183±18*
Recovery	4.0±0.3	105±8	77±6	87±2	−2.6±0.6	50±15	70±10	78±9	184±13

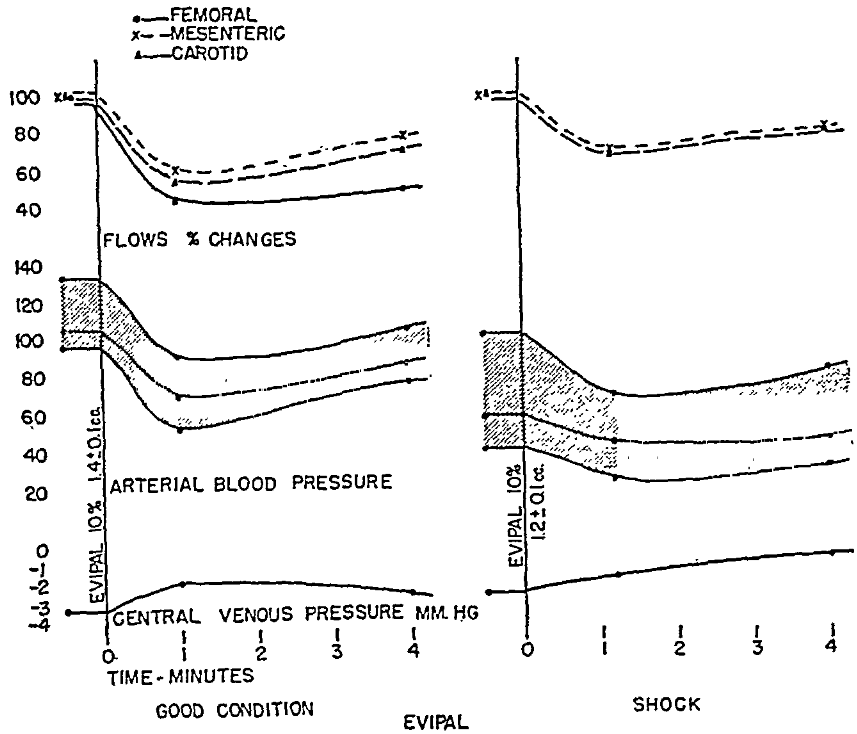


FIG. 2. ACUTE EFFECTS OF EVIPAL INJECTION ON FLOWS AND PRESSURES IN DOGS IN GOOD CONDITION AND IN SHOCK

Points are averages of pressures in mm. Hg or of flows in percentages of control values before injection (see Tables I and II).

since, at this time, our primary interest is in qualitative effects, we have included these data. We frequently checked flow changes in an artery with simultaneous records from the corresponding vein (Figure 3) and since the correlation was good, we regard our recorded changes as accurate in direction and approximately correct as to percentage, and not due to changes in pulse wave form or artefacts which would scarcely be likely to affect both artery and vein alike.

Variations in anesthesia depth. The results of deepening the anesthesia with evipal in animals, both in good condition and in shock, can be summarized very briefly (Tables I, II, Figure 2): reduction in systolic, diastolic, and mean arterial pressures; rise in central venous pressure;

TABLE II

Sodium evipal—dog in shock

Means with standard errors

	Minutes after injection	Arterial blood pressure: Systolic	Arterial blood pressure: Diastolic	Arterial blood pressure: Mean	Venous pressure	Flows in percentages of control level: Femoral artery	Flows in percentages of control level: Carotid artery	Flows in percentages of control level: Superior mesenteric or portal vein	Heart rate
			mm. Hg		*mm. Hg*				*per minute*
Control—before injection		102±9	41±4	58±4	−2.6±0.4		100	100	216±10
Sodium evipal—10 per cent solution 1.2±0.1 cc. i.v.									
Maximum effect	1.2±0.2	69±7	24±3	31±4	−2.6±0.4		67±10	71±5	219±19
Recovery	4.0±0.5	82±9	31±4	45±5	−0.7±0.9		80±4	81±6	226±8

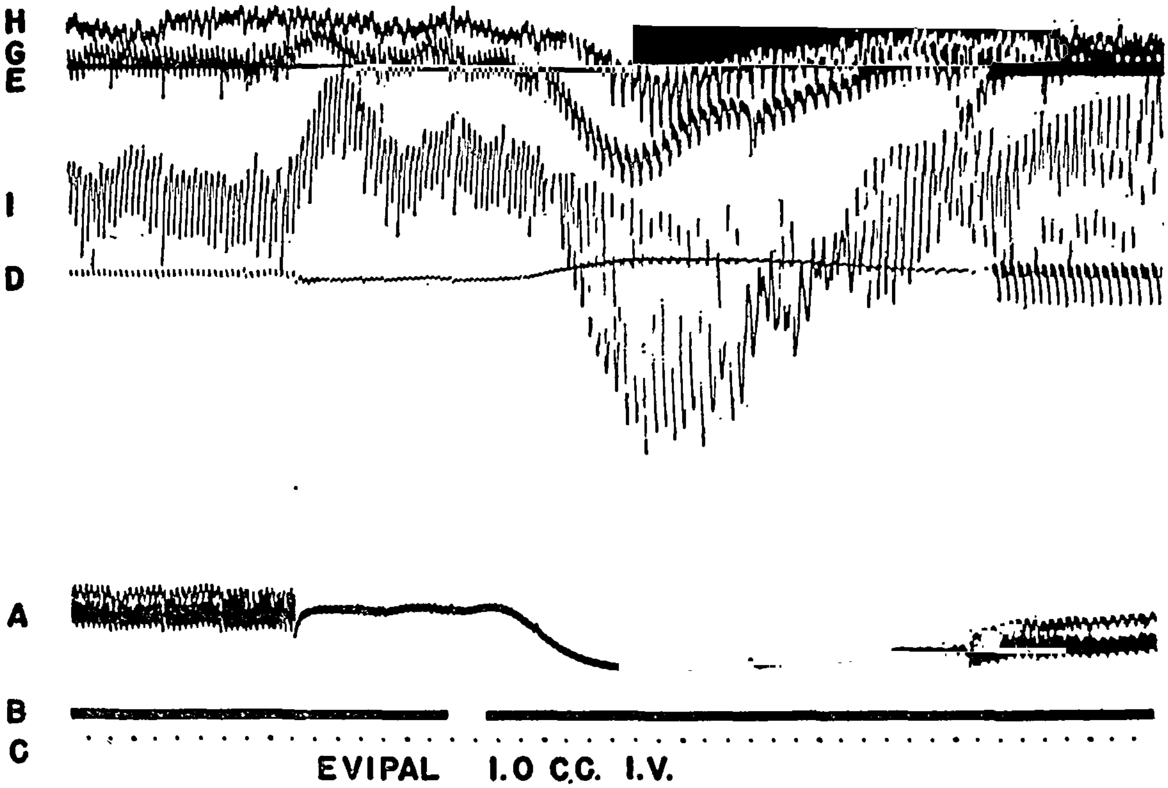


FIG. 3. OPTICAL TRACING OF ACUTE EFFECTS OF INJECTION OF EVIPAL, 10 PER CENT SOLUTION, 1.0 CC. INTRAVENOUSLY, AT BREAK IN ARTERIAL PRESSURE BASE-LINE

Key to figures in all tracings. A = Arterial blood pressure, either undamped or damped. B = Arterial pressure base line. C = Time: small marks, 10 seconds; large marks, 1 minute. D = Central venous pressure, either damped or undamped. E = Venous pressure base-line. F = Femoral artery flow. G = Carotid artery flow. H = Superior mesenteric vein flow. I = Jugular vein flow. J = Flowmeter base-line.

diminution in flow through the carotid, femoral, and mesenteric vascular beds, as recorded either in the artery or in the vein; no consistent changes in heart rate. Impedance calculations were not made with the evipal flow data, since these flow values were not considered dependable in a quantitative sense.

The mean arterial pressure changes found here confirm those described by Das (9) and others. The maximum effect on the circulation usually occurred about a minute after the intravenous injection, with all components recovering gradually thereafter over a period of many minutes, oftentimes not reaching again the original control levels.

The only important difference between the acute effects of evipal injections in animals in good condition as compared with animals in shock was that in shock, recovery from the effects took considerably longer than in animals in good condition and was almost always incomplete. These effects and differences are summarized in Tables I and II and charted in Figure 2 with time relationships shown. A typical record of simultaneous flow and pressure changes is shown in Figure 3.

Progressive changes with shock. As shock progressed (Figures 11, 12) with the subject under light evipal anesthesia, the systolic, diastolic, and mean arterial pressures fell as expected. Under evipal, a curious difference from the other agents was apparent: Starting from an initial normal pulse pressure, as shock progressed, the pulse pressure became progressively larger as a result of the diastolic pressure falling more rapidly than the systolic. The widest pulse pressure was found usually with a mean arterial pressure of 40 to 50 mm. Hg. During the agonal stage, the pulse pressure narrowed rather rapidly.

Ether

Variations in anesthesia depth. When flow and pressure were recorded in animals in good

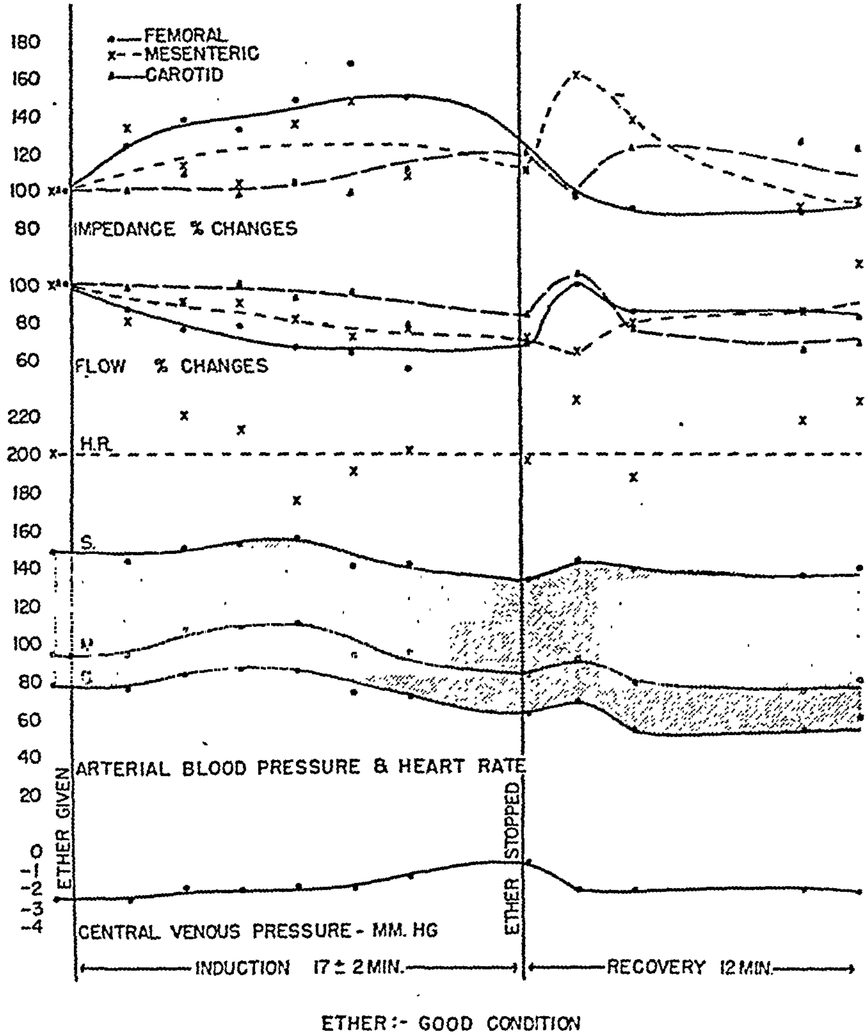


FIG. 4. PRESSURE, FLOW, AND IMPEDANCE CHANGES IN DOGS IN GOOD CONDITION UNDER ETHER ANESTHESIA, WHEN CARRIED FROM A CONTROL POINT UNDER VERY LIGHT ANESTHESIA TO A DEEP LEVEL AT OR CLOSE TO RESPIRATORY FAILURE

The actual time of ether administration varied somewhat from dog to dog, and points have been charted along the abscissa according to the fraction of the time elapsed between the point of starting ether and the time of termination. Abscissae during the recovery period are taken on a straight time basis (see Table III).

condition under light ether anesthesia, and the level of anesthesia then deepened by giving ether until respiratory failure was approached or achieved, the following changes were observed (Table III, Figures 4, 6A): The central venous pressure progressively rose; the systolic, diastolic, and mean arterial pressures usually rose somewhat initially and then fell; blood flow in the femoral and carotid arteries and in the superior mesenteric vein tended to diminish; the hydraulic impedance in these vascular beds increased. The reduction in flow with deepening ether anesthesia confirms a similar observation by Mann, Herrick, Essex, and Baldes (10). The rise in arterial blood pressure found early on deepening the anesthesia probably corresponds to a similar rise described by McAllister and Root (11).

In animals in good condition, discontinuance of the ether supply brought about the following changes: The central venous pressure fell rather rapidly towards the original level. The arterial

pressure recovered rapidly, often rebounding initially above the level at which it finally stabilized. Flow in femoral and carotid arteries usually showed transient increases for a minute or more before tapering off at a stable level; conversely, the mesenteric vein flow tended to show a decrease followed by recovery (Figures 4, 6B). The hydraulic impedance of femoral and

TABLE III

Ether—dog in good condition

Means with standard errors

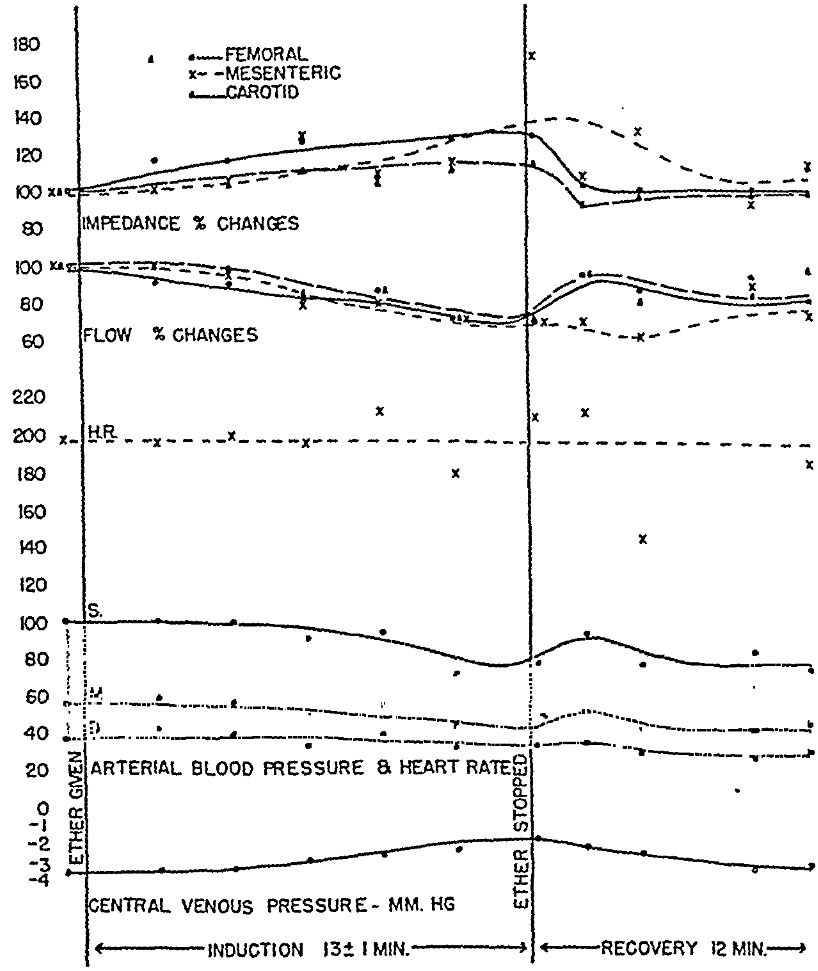
	Arterial blood pressure			Venous pressure	Flows in percentages of control level			Heart rate
	Systolic	Diastolic	Mean		Femoral artery	Carotid artery	Superior mesenteric or portal vein	
		mm. Hg		*mm. Hg*				*per minute*
Control Point 1	148±12	78±8	94±9	−2.6±0.1	100±0	100±0	100±0	200±10
Ether started								
2	143±12	76±7	94±10	−2.6±0.3	87±13	97±4	80±15	
3	150±13	83±6	107±8	−2.0±0.3	76±10	91±3	92±5	221±40
4	152±10	86±5	107±6	−2.1±0.3	77±11	100±8	91±6	203±2
5	155±9	85±3	110±5	−2.0±0.3	67±9	93±5	82±7	176±28
6	140±8	73±1	93±3	−2.1±0.1	64±11	96±7	73±12	191±10
7	141±8	71±1	94±6	−1.5±0.3	55±15	78±9	77±6	202±19
Ether stopped								
8	133±2	62±8	83±7	−0.7±0.4	69±9	84±18	72±9	197±15
9	143±8	68±5	91±6	−2.2±0.7	100±13	106±23	64±13	229±7
10	138±8	53±8	78±7	−2.2±0.4	85±11	76±17	79±21	187±17
11	134±7	53±7	74±7	−2.1±0.3	85±10	65±13	85±7	218±14
12	139±3	60±14	80±11	−2.2±0.3	82±11	68±12	111±30	228±18

TABLE IV

Ether—dog in shock

Means with standard errors

	Arterial blood pressure			Venous pressure	Flows in percentages of control level			Heart rate
	Systolic	Diastolic	Mean		Femoral artery	Carotid artery	Superior mesenteric or portal vein	
		mm. Hg		*mm. Hg*				*per minute*
Control Point 1	100±4	37±4	56±3	−3.5±0.3	100±0	100±0	100±0	197±13
Ether started								
2	99±6	42±4	58±6	−3.4±0.6	90±8	98±7	100±3	195±15
3	99±3	39±4	56±4	−3.3±0.4	90±5	97±3	96±7	198±15
4	90±4	32±4	50±3	−2.9±0.4	83±9	84±5	77±8	194±20
5	93±5	38±6	54±4	−2.6±0.4	86±5	86±6	79±10	212±21
Ether stopped								
6	76±4	32±2	40±3	−1.8±0.5	68±8	69±7	67±17	208±32
7	92±12	34±7	50±7	−2.2±0.6	94±13	94±14	69±16	210±27
8	75±8	28±3	40±3	−2.6±0.7	85±9	79±6	60±12	142±18
9	82±9	26±3	40±2	−3.4±0.3	92±8	82±6	87±9	
10	73±8	29±4	43±5	−3.2±0.4	78±9	95±8	71±7	183±39



ETHER:- SHOCK

FIG. 5. PRESSURE, FLOW, AND IMPEDANCE CHANGES IN DOGS IN SHOCK WHEN CARRIED FROM LIGHT ETHER ANESTHESIA TO A DEEP LEVEL BY ADMINISTRATION OF ETHER

Layout and interpretation of chart as for Figure 4 (see Table IV). The series of points immediately before "ether stopped" is omitted from Table IV.

ırotid vascular beds tended to decrease im-ediately after removing the ether, and then rise again towards original levels. The esenteric impedance, conversely, tended to rise hile the others fell, and then to fall again owards the starting value.

In shock (Table IV, Figure 5), the effects of eepening the anesthesia were comparable to the receding except that: The rise in central venous ressure was usually not as high as previously. The initial rise in arterial pressure was usually acking and the subsequent fall was of greater nagnitude than before, with a considerable lecrease in pulse pressure (Figure 5).

In shock, the sequence in recovery of a light level of anesthesia was almost the same as in good condition, except that events were drawn out a little in time. The heart rate showed no consistent or significant changes with administration of ether or recovery from it. Charts of trends of pressure and flow are shown in Figures 4 and 5, and actual records of changes with varying depth of ether in Figure 6. Individual variations were considerable. In some of our runs, the initial rise in blood pressure was absent (Figure 6A). Similarly, the reciprocal flow changes in carotid and femoral arteries on the one hand and mesenteric vein flow on the other,

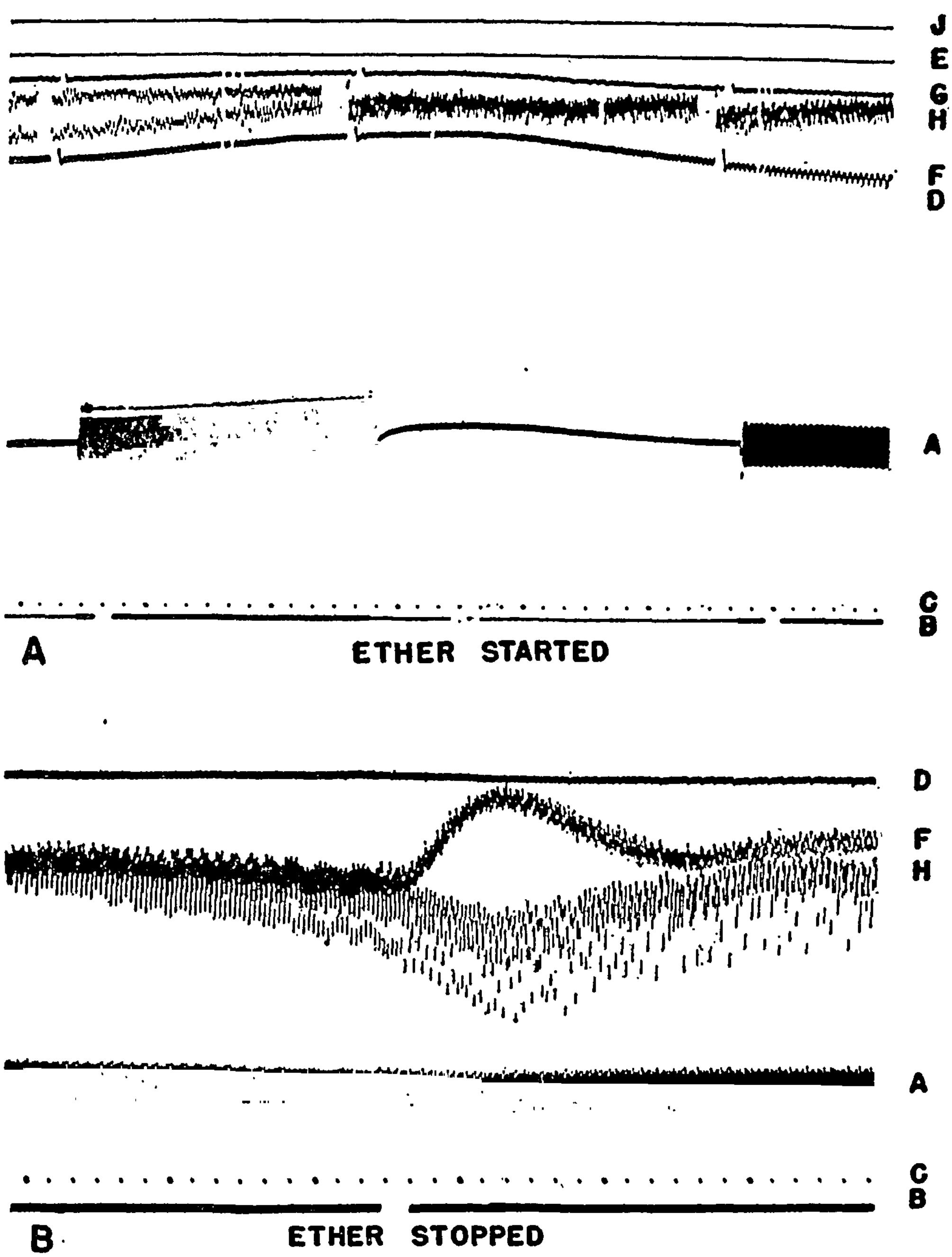


FIG. 6. A. PRESSURE AND FLOW CHANGES BEFORE AND AFTER ADMINISTERING ETHER (COMPARE WITH MEANS IN TABLE III)

The first part of the record is taken while the animal is becoming lighter from a previous deepening with ether. Trends in flows and arterial pressures are upwards, and in venous pressure, downwards. With the start of ether administration, a downward trend in flows and arterial pressure appears and venous pressure starts to rise. In this case, the arterial pressure does not show an initial rise early in the course of deepening, as many of our animals do, and as shown in Figure 5.

B. CHARACTERISTIC FLOW AND PRESSURE CHANGES AFTER STOPPING ETHER

After turning off the ether, a downward trend in arterial pressure is reversed, and a rising venous pressure starts to fall. Mesenteric vein flow shows evidence of a brief episode of vasoconstriction, and the femoral artery flow, a simultaneous phase of vasodilation, followed by a trend towards original levels. Key to tracings are as given in Figure 3.

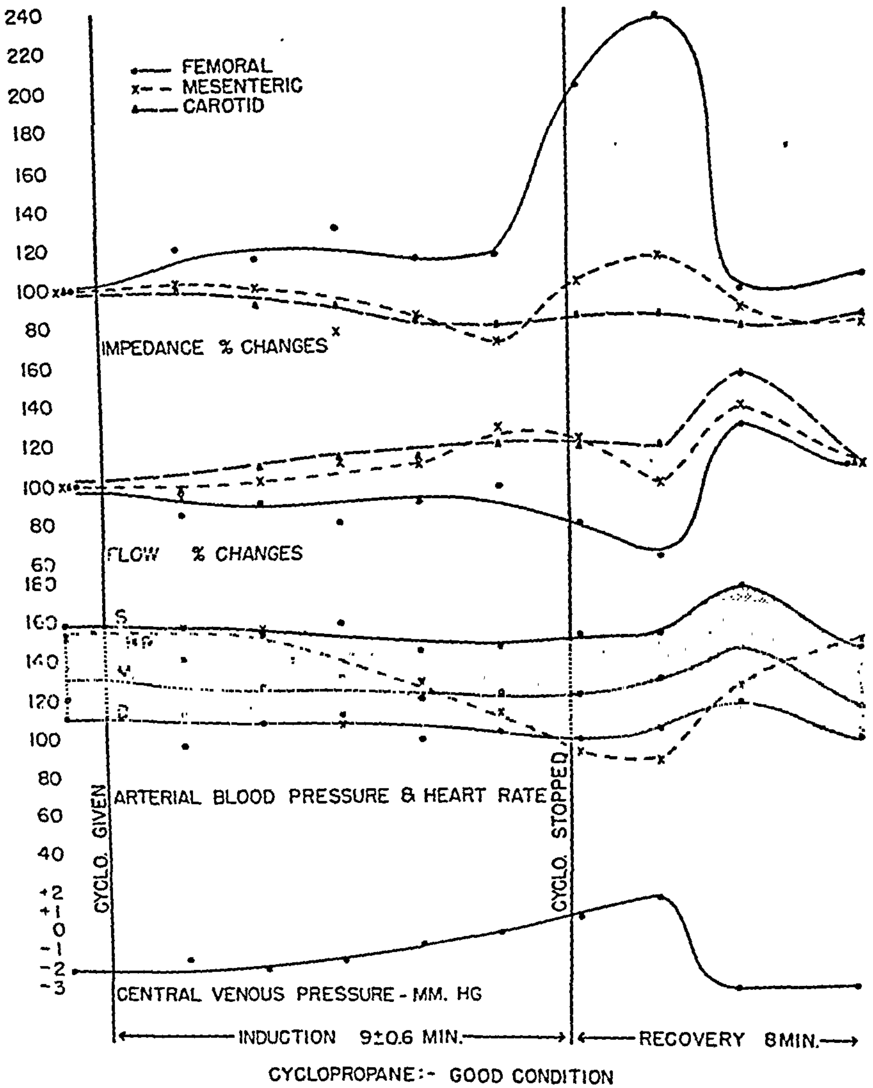


FIG. 7. PRESSURE, FLOW, AND IMPEDANCE CHANGES IN DOGS IN GOOD CONDITION UNDER CYCLOPROPANE ANESTHESIA, WHEN CARRIED FROM A CONTROL POINT UNDER VERY LIGHT CYCLOPROPANE ANESTHESIA TO A DEEP LEVEL CLOSE TO RESPIRATORY FAILURE

Abscissa and general considerations are as for Figure 4 (see Table V).

found usually after removing the ether, were sometimes absent entirely, with all three flows recovering steadily and at about the same rate.

Episodes of deliberate deepening of ether anesthesia to a point near respiratory failure seemed to be deleterious, as flows and pressures seldom recovered to original levels (see Figures 4 and 5). Several such episodes of deepening sometimes sufficed to put the animal into a shock-like state, even without any considerable blood loss. This was in contrast to the behavior of the animal under cyclopropane and is in agreement with the observations of Evans and Beecher (12) who found that dogs under ether tolerated shocking procedures less well than dogs under cyclopropane.

Progressive changes with shock. With a light level of ether anesthesia as shock progressed, the systolic, diastolic, and mean arterial pressures declined. The pulse pressure was initially much wider under ether than under cyclopropane or under evipal (Figures 11, 12), and as shock

progressed, the pulse pressure became narrower than it had been. In this case (cf. evipal), the systolic pressure fell more rapidly than diastolic. This process continued until death.

Cyclopropane

Variation in anesthesia depth. Flow and pressure records of dogs in good condition under cyclopropane anesthesia, taken at a control level

TABLE V

Cyclopropane—dog in good condition

Means with standard errors

	Arterial blood pressure			Venous pressure	Flows in percentages of control level			Heart rate
	Systolic	Diastolic	Mean		Femoral artery	Carotid artery	Superior mesenteric or portal vein	
	mm. Hg			mm. Hg				per minute
Control Point 1	159±5	111±5	121±6	−2.2 ±0.3	100±0	100±0	100±0	153±8
Cyclopropane started								
2	141±14	97±12	113±13	−1.6 ±0.5	85±8	98±4	96±7	157±13
3	153±5	108±5	127±5	−2.04±0.3	91±5	109±3	102±5	157±7
4	159±9	112±8	131±9	−1.6 ±0.5	81±8	114±9	112±8	107±14
5	145±7	99±6	120±6	−0.7 ±0.4	91±6	114±6	112±7	129±10
6	147±6	103±6	123±7	−0.1 ±0.4	99±13	120±8	129±7	113±9
Cyclopropane stopped								
7	153±12	99±10	122±10	+0.7 ±0.7	80±15	119±9	123±31	93±8
8	153±8	104±7	130±5	+1.7 ±0.5	63±9	119±7	101±11	89±5
9	177±11	118±7	146±7	−3.0 ±0.4	129±12	154±9	138±11	127±9
10	146±6	100±6	116±6	−2.8 ±0.3	108±9	108±4	109±4	150±10

TABLE VI

Cyclopropane—dog in shock

Means with standard errors

	Arterial blood pressure			Venous pressure	Flows in percentages of control level			Heart rate
	Systolic	Diastolic	Mean		Femoral artery	Carotid artery	Superior mesenteric or portal vein	
	mm. Hg			mm. Hg				per minute
Control Point 1	85±2	39±2	52±2	−3.6±0.4	100±0	100±0	100±0	168±9
Cyclopropane started								
2	94±5	42±2	58±3	−3.6±0.4	97±8	106±8	103±5	168±14
3	91±4	41±5	55±3	−2.8±0.4	91±8	105±6	106±5	181±10
4	79±4	40±3	53±3	−3.4±0.4	94±16	105±7	100±15	156±9
5	75±4	33±3	46±4	−2.1±0.4	90±9	94±8	88±9	163±15
6	85±6	42±5	56±5	−2.8±0.5	86±11	109±10	111±20	128±7
7	80±5	39±4	51±4	−2.2±0.3	75±12	97±9	87±13	143±10
Cyclopropane stopped								
8	68±4	29±3	41±4	−2.0±0.9	72±13	72±11	80±19	116±10
9	74±8	38±6	51±5	−1.4±0.4	66±11	84±10	78±13	122±9
10	79±5	36±3	50±3	−3.0±0.7	99±16	108±12	106±13	154±12
11	77±4	31±3	46±3	−3.5±0.4	87±9	96±7	107±18	172±17

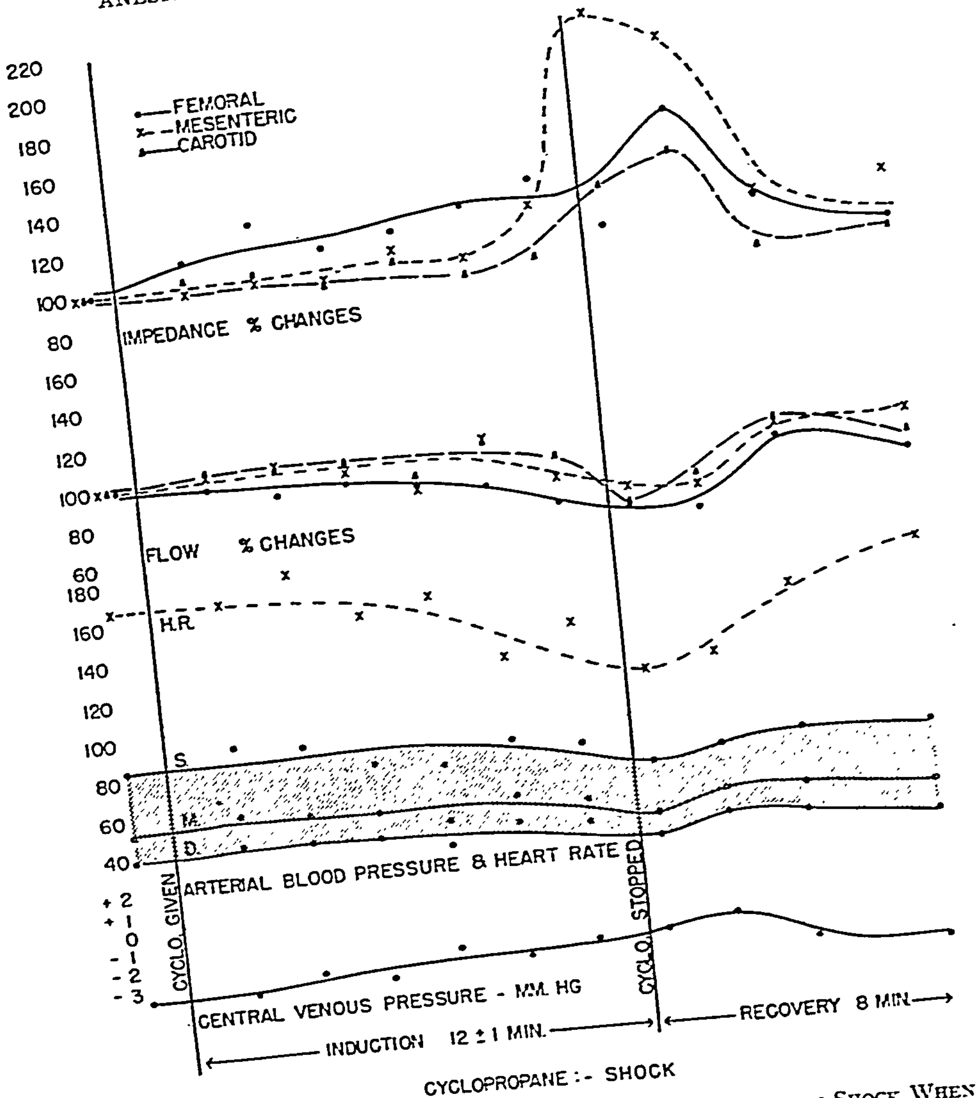


FIG. 8. PRESSURE, FLOW, AND IMPEDANCE CHANGES IN DOGS IN SHOCK WHEN CARRIED FROM VERY LIGHT CYCLOPROPANE ANESTHESIA TO A VERY DEEP LEVEL (SEE TABLE VI)

of very light anesthesia and then followed through a process of deepening with cyclopropane to a point approaching respiratory failure, in a manner similar to that described for ether, showed the following changes (Table V, Figure 7): Central venous pressure rose remarkably and progressively, reaching levels far above those attained under ether or evipal. Arterial pressure was either unaffected or showed inconstant changes. Heart rate progressively decreased. Femoral flow tended to decrease, while flow in carotid and mesenteric beds increased. Hydraulic impedance in the femoral bed usually increased and, in the carotid and mesenteric, generally decreased. The lack of a constant effect on arterial blood pressure is in agreement with Waters (13). A slowing of the heart rate under cyclopropane has often been reported previously (see Seevers and Waters (14)). In studying the effects of cyclopropane on the circulation in a heart-lung preparation (dogs), Krayer and Beecher (15) observed a striking rise in venous pressure under this agent. Volpitto, Woodbury, and Hamilton (16) reported a rise in venous pressure in man under cyclopropane.

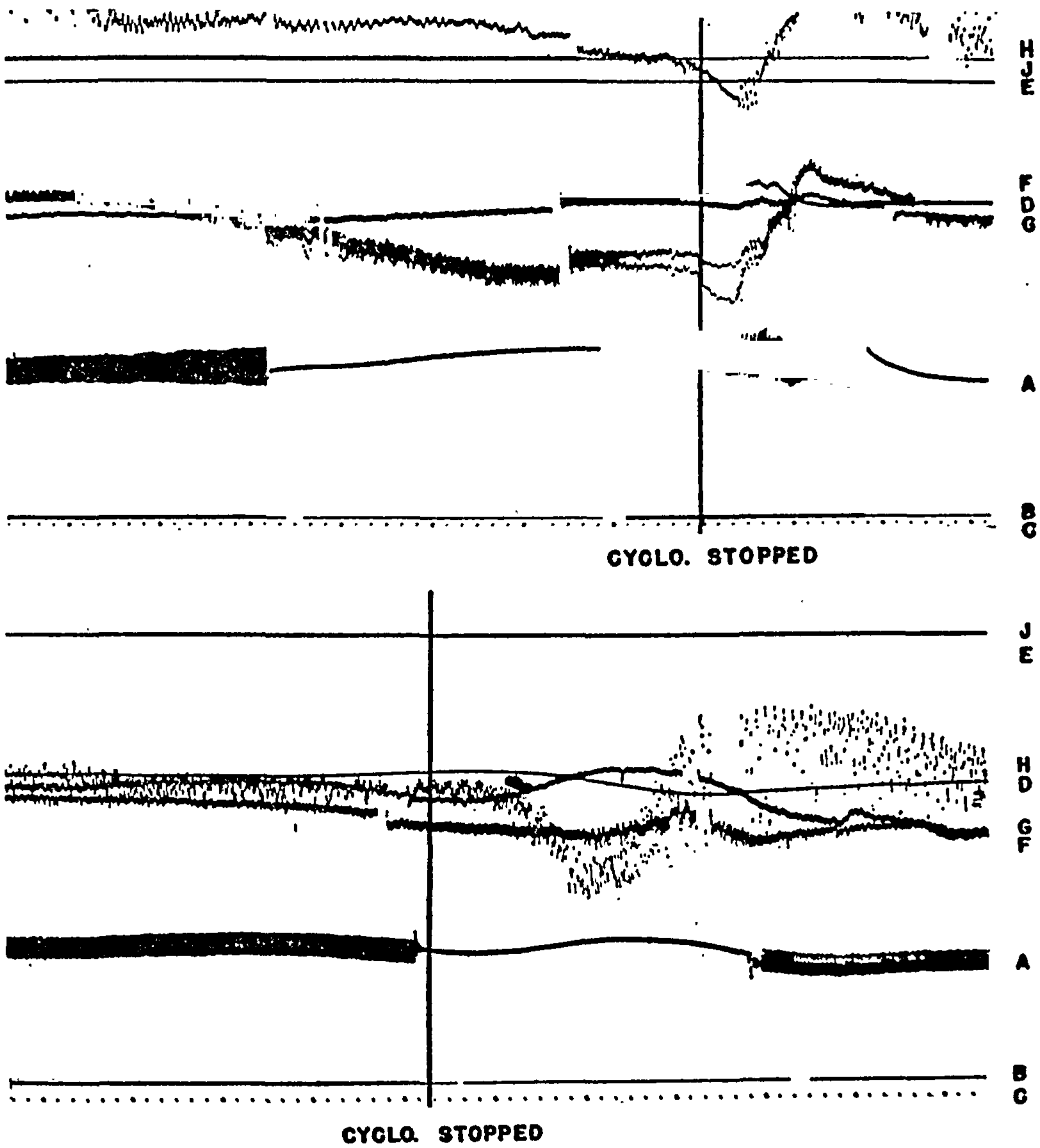


FIG. 9. PRESSURE AND FLOW CHANGES AFTER ADMINISTRATION OF CYCLOPROPANE HAD ALREADY BEEN STARTED

The 2 records show the later phases of deepening anesthesia with cyclopropane, the time of stopping the administration, and the recovery. The upper record shows a typical decrease in femoral flow, increase in carotid flow, rise in venous pressure, and slowing of heart rate on deepening anesthesia, with characteristic rebound in femoral and mesenteric flow on recovery. The marked rise in arterial pressure with deepening is atypical, and the decrease in mesenteric flow in deep anesthesia is greater than usual. The lower record shows typical rising venous pressure and constant arterial pressure on deepening, with the typical rise and fall of arterial pressure on recovery. Femoral flow changes are less marked than usual.

Upon removal of cyclopropane and on allowing the animal to recover, a rather complex series of changes characteristically occurred, as follows: The elevated central venous pressure fell rapidly to a point slightly below its original value and gradually rose to a normal level for light anesthesia. The arterial pressure sometimes fell briefly but usually approached the original condition only after a sharp rise above its final value, described previously by Brace, Scherf, and Spire (17). Heart rate was restored rapidly to normal. Femoral flow decreased sharply for a short time and then showed a phase of increase from which it diminished to a stable value. Carotid artery flow showed a less marked transient wave of increased flow, after which it fell slowly to normal. Mesenteric vein flow usually showed changes similar to those of the

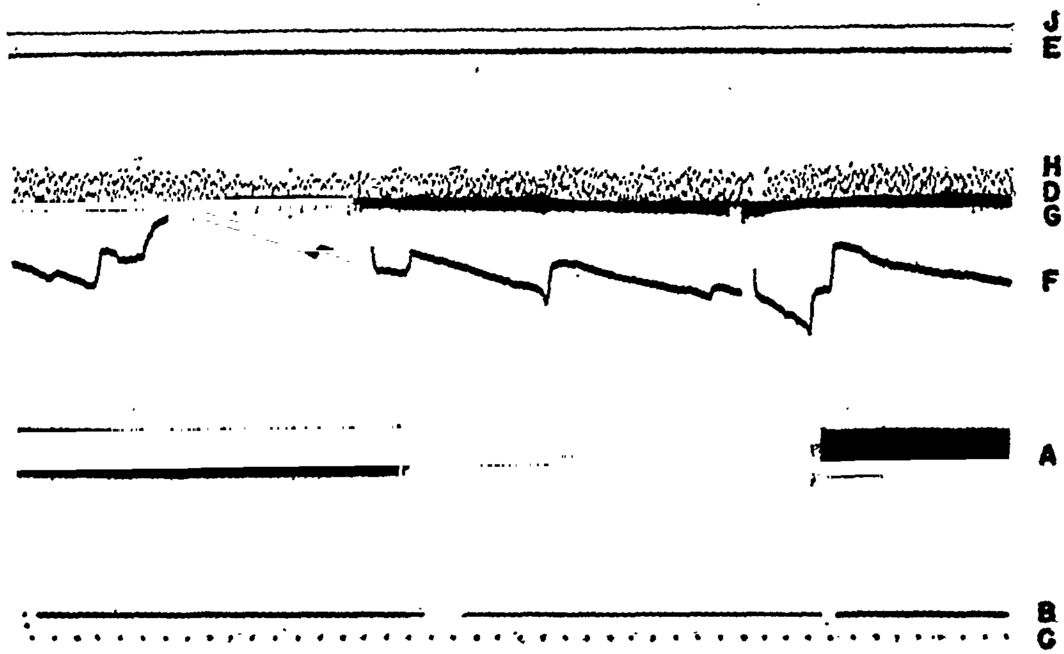


FIG. 10. OPTICAL TRACINGS OF A DOG UNDER ETHER ANESTHESIA TAKEN AT A TIME WHEN FLOW IN THE FEMORAL ARTERY WAS SHOWING IRREGULAR AND UNPREDICTABLE CHANGES, WITHOUT EVIDENCE OF COMPENSATING FLOW CHANGES IN CAROTID OR MESENTERIC VASCULAR BED, OR OF CORRESPONDING CHANGES IN CENTRAL ARTERIAL OR CENTRAL VENOUS PRESSURE

femoral, but with smaller excursions. Hydraulic impedances showed an increase followed by a decrease, a phenomenon most marked in the femoral vascular bed, less marked in the mesenteric, and questionably present in the carotid.

The rise in central venous pressure, the fall in heart rate, and the increase in carotid and mesenteric flow and the depression of femoral flow during deepening anesthesia were quite constant. Arterial pressure changes during deepening were variable; sometimes no change appeared. More often there were unpredictable phases of increased or decreased pressure.

On recovery, the fall in venous pressure was always present, but the arterial pressure sometimes fell steadily (Figure 9A) or rose and then fell (Figures 7, 9B), or changed very little. The changes in flow early in recovery were quite variable in degree; examples of some variations met are shown in Figure 9. The time relationship which the flow and pressure changes bore to each other on recovery were also quite variable. Thus, the phase of vasodilatation, characterized by the increase in femoral and mesenteric flow, occurred in some cases while the pressure was falling, as in Figure 9A, or partly during a phase of rising pressure and partly during a phase of fall, as in 9B, or entirely during a rise. Similarly, flow changes in one vascular bed were not necessarily accompanied by parallel changes in another, as in Figure 9B, where the carotid flow changes were entirely out of phase with the mesenteric and femoral.

In shock, the changes on deepening the anesthesia (Table VI, Figure 8) were comparable to those found in the animal in good condition, except that: The central venous pressure started lower and did not rise to the same high level as formerly. The arterial blood pressure was usually somewhat depressed when the anesthetic was pushed to a deep level. The heart rate was a little faster throughout than it had been during good condition. As formerly, slowing occurred with deepening anesthesia. The flow changes were directionally the same, but showed less violent swings on recovery and the phases were somewhat prolonged.

These episodes of deepening with cyclopropane, in contrast to those with ether, appeared to have no particularly lasting deleterious effects on the dogs. Flows and pressures usually returned to control levels upon recovery from deepening,

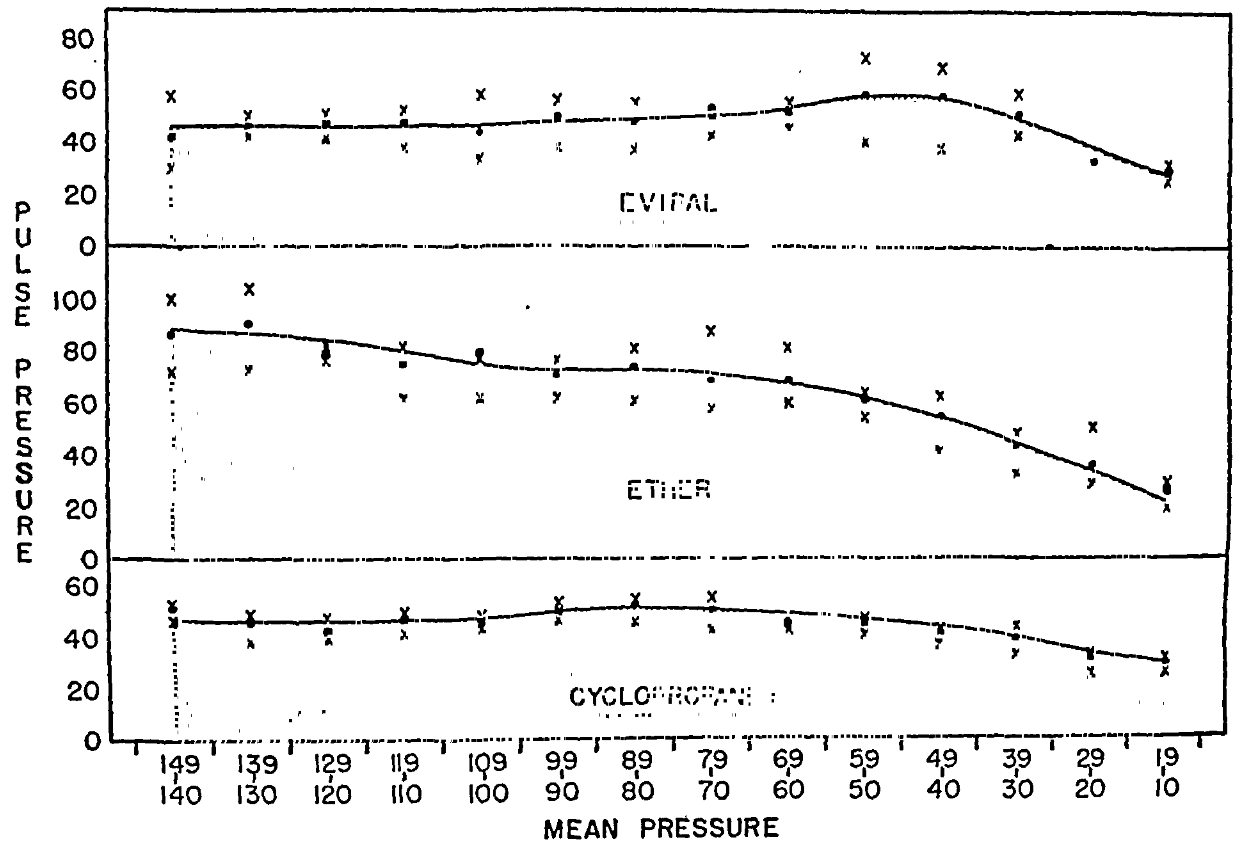


FIG. 11. PULSE PRESSURE CHANGES WITH SHOCK UNDER EVIPAL, ETHER, AND CYCLOPROPANE

The dots represent the averages of all readings within the mean arterial pressure range shown on the abscissa. The crosses denote the limits between which half of our pulse pressure readings lie, and hence give an indication of the scatter.

and in many dogs, episodes of deepening were imposed 5 or 6 times without any noticeable deterioration of the circulation of the animal.

Progressive changes with shock. With a light level of cyclopropane anesthesia, as shock progressed, the systolic, diastolic, and mean pressures declined. The pulse pressure was initially narrow, as under evipal, and remained fairly constant throughout the shock process (Figures 11, 12), with the exception that occasionally an animal showed some widening of the pulse pressure as the mean blood pressure approached the shock level; but in these cases, as the shock progressed, this would narrow again to approximately the original size.

GENERAL CONSIDERATIONS

The optical tracings (Figure 12) show an example of a further difference in the nature of the pulse wave with the 3 anesthetics. With animals in good condition under ether, the mean pressure is well below a point halfway between systolic and diastolic pressure. Under evipal and cyclopropane, the mean pressure is nearer the midpoint between the systolic and diastolic peaks.

The data obtained (Table VII) give no evidence of different flow values under ether and cyclopropane, respectively, in these 3 vascular beds; accordingly, one is tempted to surmise that the cardiac output under these 2 agents might be likewise comparable, and in fact, Blalock (18) and Robbins and Baxter (19) have shown respectively that the cardiac output is greatly increased both under ether and under cyclopropane. If this speculation be valid, one can couple it with the lower heart rate under light cyclopropane than under light ether and conclude that the difference in pulse pressure observed is, in all likelihood, due to differences in the elastic properties of the aorta and large arteries with these 2 agents. This would mean that the hydraulic capacitance[4] of the aorta and large

[4] Hydraulic capacitance refers to distensibility, in a sense comparable to electrostatic capacitance, and not to volume.

rteries is greater under cyclopropane than nder ether. In other words, the elastic tension r Hooke's law constant of the aorta should, if his view holds, be greater under ether than nder cyclopropane and it would appear to serve s a more effective damping device under cyclo-ropane than under ether. It is possible that lifferences in the time of systolic upswing might n part impair the validity of these speculations.

Rhythmic sinusoidal fluctuations in arterial blood pressure (Traube-Hering waves) were completely absent in all 25 of our dogs under ether anesthesia. They were present in 5 of 11 dogs under cyclopropane and in 14 out of 27 dogs under evipal. Although present in about half the dogs under evipal and cyclopropane, Traube-Hering waves were most persistent and of greater amplitude under evipal than under cyclopropane. If such waves were present at the time of injection of evipal intravenously, they invariably disappeared with the other acute effects of the injection, often to reappear gradually with recovery after 8 or 10 minutes.

Our records of mean central venous pressure showed in most cases a tendency towards progressive decline during the shock process, with a terminal rise. This fall in central venous pressure generally observed is at variance with the findings reported by Werle, Cosby, and Wiggers (3), who, however, did not measure the mean central venous pressure, but took their value from an arbitrary point on the venous pulse curve. The progressive venous pressure decline could be interrupted or reversed temporarily by deepening the anesthesia by administration of ether, cyclopropane, or evipal. Apart from the varying duration of the effects of deepening anesthesia with various agents, the decline of central venous pressure with shock occurred under all 3 of these substances.

The nature of the agonal changes in animals dying in hemorrhagic shock differed according to the anesthetic used. Under cyclopropane or ether, the dying animals usually showed a progressive decline in systolic peak pressures with slowing of the heart rate until complete circulatory standstill was achieved. In contrast, animals dying of shock under evipal showed a similar initial slowing of the pulse and fall in pressure, followed characteristically by a brief burst of cardiac recovery, with restoration of blood flow and transient fall in central venous pressure. The recovery might last for 1 or 2 minutes and then fade out with resulting final cardiac standstill, or, in some instances, regress only to flare up again. The cardiac slowing before the reactivation observed under evipal was in some dogs not marked, but in about half (12 out of 26) of our animals under evipal, the heart slowed to complete standstill for a period of from 10 seconds to a minute, and then recovered as described for a minute or two. Such "recoveries" were not observed under ether or under cyclopropane.

When arterial and central venous pressures were measured carefully from the same zero base-line at the level of the opening of the vena cava, as explained previously, even in an animal dead from hemorrhagic shock, the arterial pres-

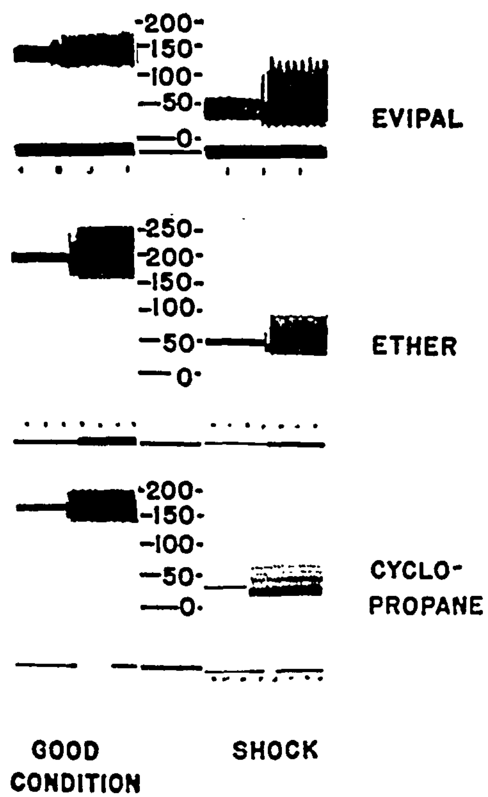


FIG. 12. OPTICAL ARTERIAL BLOOD PRESSURE, MEAN AND FULL PULSE PRESSURES WITH THE ANIMAL IN GOOD CONDITION AND IN SHOCK, UNDER EVIPAL, ETHER, AND CYCLOPROPANE

Each pair shows records from the same animal, taken at different times during an experiment.

TABLE VII

Tabulation of body surface of dogs, and flows and impedances, corrected for body surface, in dogs under light cyclopropane and under light ether anesthesia, in good condition and shock

Body surface	Femoral flow	Carotid flow	Mesenteric flow	Femoral impedance	Carotid impedance	Mesenteric impedance
sq. m.	*cc. per second per sq. m.*	*cc. per second per sq. m.*	*cc. per second per sq. m.*			
Cyclopropane—dog in good condition						
0.66±0.02	0.43±0.18	1.4 ±0.3	4.4±0.6	630±150	130±15	38±9
Ether—dog in good condition						
0.73±0.02	0.37±0.08	1.3 ±0.2	3.5±1.0	460±230	120±40	39±7
Cyclopropane—dog in shock						
0.68±0.02	0.20±0.07	0.51±0.01	1.8±0.3	520±110	130±25	36±7
Ether—dog in shock						
0.70±0.03	0.29±0.08	0.66±0.08	1.8±0.3	420±200	96±12	47±8

sure never fell to zero, but came to rest with the venous pressure at 2 to 5 mm. Hg above the zero level mentioned. No significant differences in heart rate changes between the 3 anesthetic agents were observed as shock progressed.

As one can readily see from the flow changes recorded in Figures 6 and 9, femoral, carotid, and mesenteric flows may not behave alike when all 3 are recorded simultaneously. Certain differences in the reactions of the femoral flow from those of the carotid and mesenteric show likewise in the chart of the averages under cyclopropane in animals in good shape (Figure 7). It is further apparent from the records that there is no consistent correlation of vasoconstriction or vasodilatation in any of these 3 vascular beds with changes in arterial blood pressure or in central venous pressure.

Spontaneous changes of considerable magnitude have been recorded in one or another of these vessels, without any evidence of compensatory changes in the other beds, or any repercussions in systemic pressure. An example of such changes is seen in Figure 10, where femoral flow shows considerable spontaneous and totally irregular flow changes without any sign of corresponding fluctuations in carotid or mesenteric flows or in pressures. Regular phasic flow changes of a similar nature have been recorded. In our experience, such phasic or irregular flow changes have been met with most frequently in the femoral artery. The flow in the carotid and mesenteric vessels has appeared to be more stable. These effects have been observed under all 3 of the anesthetic agents.

Insofar as the primary anesthetic effects go, the 3 agents considered here have great clinical value, notwithstanding considerable variation in their particular fields of usefulness. Pertinent to the problem of determining the best anesthetic to use in men in shock is a comparison of the secondary effects of these agents, particularly on the circulation. In this connection, several facts stand out; for example, our findings suggest that cyclopropane is tolerated better by dogs in shock than is ether or evipal. This confirms the previous observation of Evans and Beecher (12). It may in part be due to the observed fact that the carotid and mesenteric flows and the arterial pressure were preserved well even when the cyclopropane was pushed to a deep level of anesthesia. This was true with cyclopropane in animals in shock as well as in good condition; but was not the case with ether or evipal. From this, one might generalize that cyclopropane affords a greater margin of safety with respect to blood pressure and blood flow preservation in essential vascular beds than do the other 2

agents. These findings in dogs suggest the desirability of searching for a similar relationship in man. The significance of the extraordinary rise of venous pressure under cyclopropane is not clear from our experiments, and should be studied further. In evaluating cyclopropane for use in wounded men, one must not lose sight of a factor not included in the present study, namely, the well-recognized tendency of cyclopropane to sensitize the heart to stimuli which produce ventricular fibrillation. This accounts for a disturbing number of clinical deaths.

As well recognized clinically and as shown in this and other experimental studies, anesthesia may be deleterious in shock. Since this is the case, the briefer the period of anesthesia, the better it is for the patient; accordingly, the rapid induction of cyclopropane anesthesia and the swift recovery from it offer a considerable advantage over ether, in this respect.

Under evipal, the tendency of the central venous pressure to rise, and the usual failure of arterial blood pressure and flow to return to original values after fairly small injections of evipal, support the view that this agent had best be reserved for brief anesthesia in subjects in good condition.

SUMMARY

The effect on the circulation of 3 representative anesthetic agents has been studied in dogs with the purpose of obtaining objective information as to the best choice of anesthetic agent for use in seriously wounded men. The influence of varying depths of anesthesia on heart rate, on systolic, diastolic, and mean arterial blood pressure, on central venous pressure, and on blood flow in 3 vascular beds, femoral, carotid, and mesenteric, has been studied in dogs in good condition and in shock (hemorrhage).

Sodium evipal effects were as follows: Deepening the anesthesia of subjects in good condition and in shock was followed in both instances by reduction in systolic, diastolic, and mean arterial pressures, by rise in central venous pressure, by diminution in flow through the carotid, femoral, and mesenteric vascular beds. There were no consistent changes in heart rate. The maximum effect on the circulation usually occurred about a minute after the intravenous injection. All components thereafter gradually approached their original levels; but when shock was present, the recovery process was slower than formerly and the original levels were not usually attained.

Under evipal, as shock progressed, diastolic blood pressure fell faster than systolic, giving a pulse pressure which widened progressively until the terminal stages.

Ether effects were as follows: With the subjects in good condition, deepening of the anesthesia was associated with a progressive rise in venous pressure; a slight initial rise in systolic, diastolic, and mean arterial pressure, followed by a fall; a diminution in blood flow in the femoral and carotid arteries and in the superior mesenteric vein, with a rise in the hydraulic impedance in these vascular beds. Discontinuance of the ether supply was followed by a rather rapid fall in venous pressure towards the original level; arterial pressures, flows, and impedances recovered rapidly.

With the subject in shock, the effects of deepening the anesthesia were similar to those in animals in good condition. During recovery, events were comparable but somewhat drawn out. An important difference from cyclopropane was evident here: Repeated deepening of the ether anesthesia was plainly deleterious; it hastened the development and increased the degree of shock, as measured by the increasing inability of the circulatory components to return to their preceding levels. As shock progressed under ether, the systolic blood pressure fell more rapidly than the diastolic, giving a continuously narrowing pulse pressure. The pulse pressure in dogs in good condition under ether was much wider than under evipal or cyclopropane.

Cyclopropane effects were as follows: With the subjects in good condition, on deepening the anesthesia, the central venous pressure rose remarkably and progressively. Arterial pressure showed inconstant changes. Heart rate progressively decreased. Femoral flow usually decreased, while that in the carotid and mesenteric beds increased; the hydraulic impedance changes were the inverse of the respective flows. Repeated deepening of anesthesia with cyclopropane, in contrast to ether, appeared to have no particularly lasting deleterious effects. In general, the changes on deepening the cyclopropane

anesthesia in dogs in shock were comparable to those observed with the animal in good condition. A complete series of flow and pressure changes on recovery from deep cyclopropane anesthesia was observed and described. As shock progressed under cyclopropane, systolic and diastolic blood pressure fell at about the same rate, without a significant change in pulse pressure, until terminal stages were reached.

In so far as one can judge from these experimental observations on dogs in shock as well as in good condition, cyclopropane might offer a wider margin of safety for anesthesia in the seriously wounded than ether or sodium evipal with respect to blood pressure and blood flow through essential vascular beds.

APPENDIX—A HEATED THERMOCOUPLE FLOWMETER[5]

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(*From the Departments of Pharmacology and Anatomy of the Harvard Medical School*)

This appendix describes a flowmeter for recording blood flow in cannulated vessels on anesthetized heparinized animals. The instrument records continuously and automatically, and is sufficiently simple in operation to allow flow to be measured in 3 separate vessels simultaneously, without necessitating the exteriorization of blood vessels or the use of hydraulic by-passes. It has been used in vessels ranging in size from the femoral of a cat to the portal vein of a dog. It could be installed in vessels either larger or smaller than those mentioned. The method appears to be worth publishing for 3 reasons: first, because it has proved to be practical and useful in a rather extensive series of experiments, as shown above; second, because its design eliminates or reduces many of the errors present in the thermostromuhr of Rein (20) and of Baldes and Herrick (21) (see Gregg *et al.* (22), Shipley *et al.* (23)); and third, because its development and use have brought to light some basic limitations and sources of inaccuracy in thermal methods of blood flow recording which have not been adequately pointed out, and which could profitably be added to the list discussed by Gregg (22) and by Shipley (23) and coworkers.

Construction

Heater. We have employed, in a modified form, the hot wire principle used by A. V. Hill (24), Anrep and coworkers (25), and Machella (26). This method depends on the fact that the temperature of a wire immersed in a moving stream of fluid or gas and heated by a constant current will depend on the rate at which heat is taken away from it by the moving stream, which in turn will be a function of velocity flow past the wire. The principle differs from that used by Rein (20) and by Baldes and Herrick (21), who heated a moving stream of blood between the 2 junctions of a thermocouple, external to a blood vessel.

The hot wire was a piece of nichrome wire 0.0015 inch (0.0381 mm.) in diameter and about 2 mm. long, with a resistance of about 2 ohms, mounted rigidly in a cannula so as to be bathed by the moving stream of blood. The nichrome wire was heated by direct current drawn from a storage battery.

Thermocouple. Instead of recording the temperature changes in the wire by following changes in its resistance while it is connected as one arm of a bridge, we have soldered to it one junction of a thermocouple, in a manner somewhat like that used by Gibbs (27) and by Schmidt and Walker (28) and as recommended by Burton (29). The other junction of the couple was mounted on the opposite side of the same cannula, 1 to 4 mm. upstream, so as to be kept as close as possible to blood temperature, and so as to be unaffected by blood heated while passing close to the hot wire. The thermocouple was in series with a moving coil galvanometer which registered the difference in temperature between the 2 junctions immersed in the blood. The deflections of the galvanometer were recorded optically.

The thermocouples were of wire 0.0015 inch (0.038 mm.) or 0.0031 inch (0.0794 mm.) in diameter. Three combinations of metals were tried: iron-constantan, copper-constantan, and chromel P-constantan. Iron-constantan couples were subject to breakage from corrosion. We were unable to establish any clear-cut preference between the other 2 combinations. The fine thermocouple wires were soldered to large (No. 28) copper leads, and these points of soldering were kept close together (but well insulated from each other) and enclosed in a small silver tube so as to keep them as nearly as possible at the same temperature and thus avoid stray thermal currents arising from this extraneous pair of junctions.

Joining of heater and thermocouple. The hot junction of the thermocouple was soldered to a point midway along the length of the nichrome heater in such a way as to prevent the thermocouple circuit from being in parallel with any part of the hot wire. In a satisfactory junction, the heating current through the nichrome could be reversed without appreciably changing the deflection of the galvanometer in series with the couple.

Fashioning of hot and cold tips. The 4 wires leading to the hot junction (the 2 thermocouple wires and the 2 portions of the nichrome heater on each side of the junction) were then folded back so as to form a narrow tent or pyramid with the junction at the apex (Figure 13), and covered with a polymerizing phenolic insulating varnish (such as Bakelite) in which powdered quartz had been suspended in order to increase thermal conductivity. The 2 wires at the cold junction were similarly fashioned into a compact tip and insulated.

[5] The development of this instrument was aided by a grant from the Milton Fund of Harvard University.

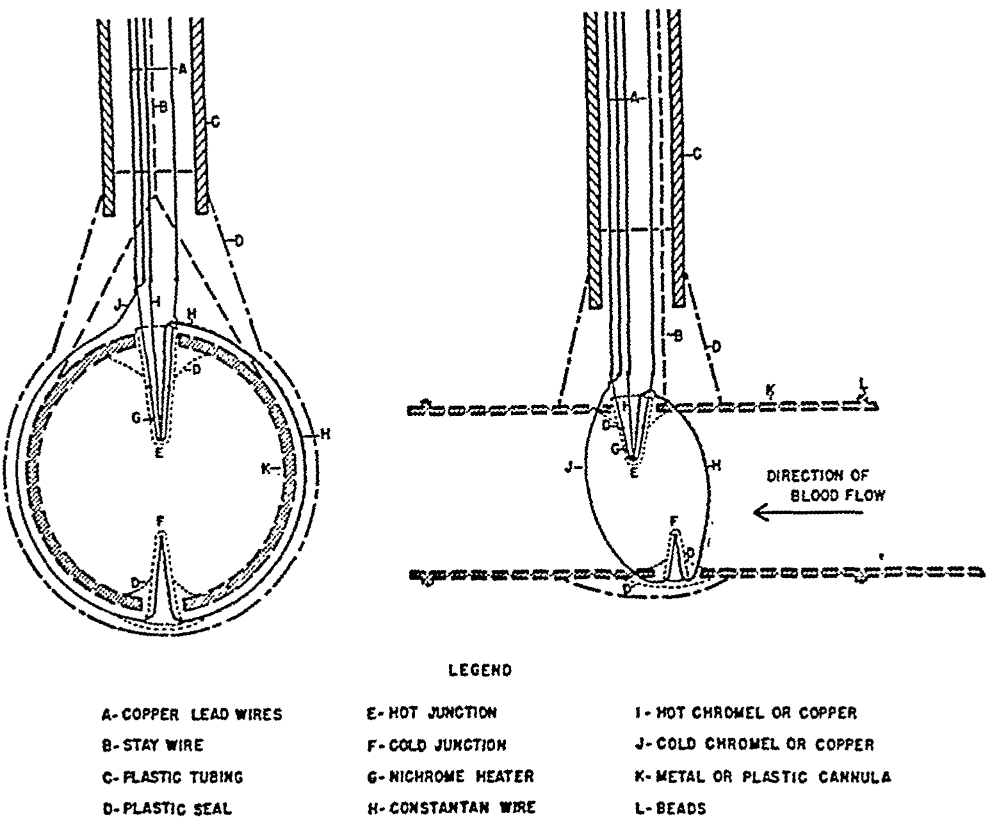


FIG. 13. DIAGRAMMATIC CROSS SECTION AND LONGITUDINAL SECTION OF FLOWMETER CANNULA WITH THERMOCOUPLE TIPS MOUNTED IN POSITION

Mounting in cannulae. The hot and cold tips connected to the lead wires were then mounted permanently and rigidly in a metal or lucite cannula, machined so as to fit conveniently into the vessel in which flow was to be measured. Metal was found to be superior to lucite because of its higher thermal conductivity. The tips were mounted as shown in Figure 13, each in a hole drilled through the wall of the cannula, with the actual thermal junctions projecting equally about one-third of the diameter into the lumen of the cannula, and with connecting wires passing around outside the cannula, as shown. The tips were fastened in place with a polymerizing insulating varnish which also provided a continuous plastic lining to the cannula.

In order to avoid possible error from galvanic currents arising from salt action on 2 different wires or from the presence of a saline bridge between the hot and cold junctions or the wires looping around the cannula externally, all wires were well covered by a suitable non-hygroscopic polymerizing resin.

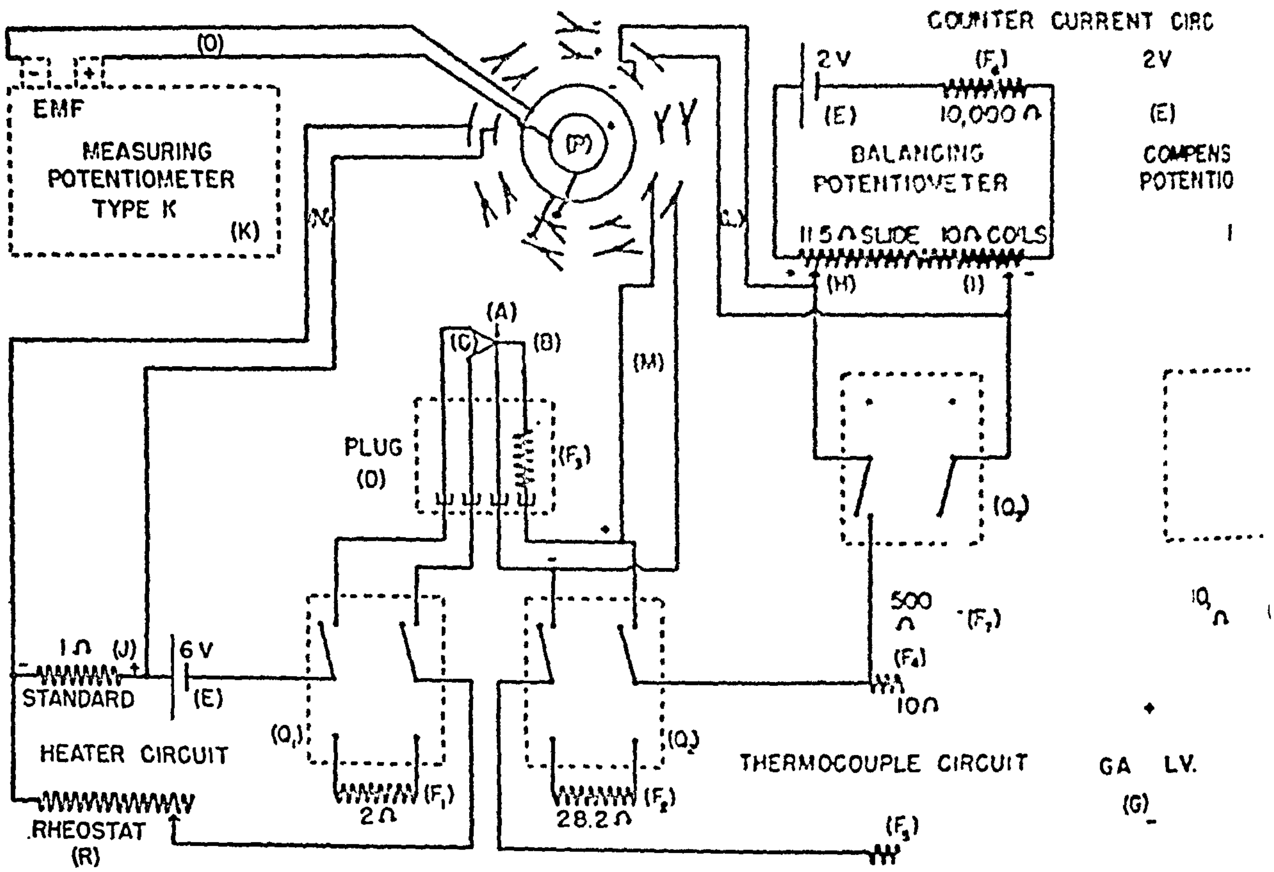
In order to relieve the electrical lead wires of mechanical stress, a steel stay wire was looped around the cannula and soldered or otherwise fastened to it. The lead wires were anchored securely to the stay wire near the cannula, and enclosed in stiff insulation for a distance of 2 or 3 cm. from the cannula, in order to spare the fine wires from breakage from repeated flexion. The stay wire was insulated and the lead wires wound around it in a loose spiral, anchored with insulating varnish, and jacketed with flexible plastic tubing. Leads about 100 cm. long were found convenient. All electrical wires were carefully insulated from the cannula and stay wire and from each other, except at the hot and cold junctions.

Provision for interchangeability. The 4 lead wires from each cannula were soldered at their free ends to a plug which could be connected interchangeably with any of 3 equivalent circuits. The free end of the stay wire was fastened to the plug in such a way as to take up tension before the electrical lead wires were pulled taut.

The resistance of each completed thermocouple was measured, and a supplementary manganin resistance coil (Figure 14, F_3) made up and mounted in the plug, the resistance of this coil being sufficient in the case of each thermocouple to bring the total resistance of the 2 elements to the same figure for all couples. In our thermocouples, the total resistance was 28.2 ohms.

Circuit. For simultaneous recording in 3 blood vessels, 3 approximately equivalent circuits were constructed as follows:

The nichrome heater (Figure 14, C) was connected through the plug and one end of a double-pole, double-throw switch (Q_1) to a low-discharge storage battery, a 1 ohm standard resistor, and a rheostat for adjusting the heater current, all in series. The current was measured



LEGEND

(A) HOT JUNCTION
(B) COLD JUNCTION
(C) NICHROME HEATER WIRE
(D) 12-PRONG JONES PLUG WITH CONTACTS SHORTED INTO 4 GROUPS OF 3 EACH
(E) WILLARD LOW DISCHARGE STORAGE CELLS (TYPES DH-5-1 OR DD-5-3)
(F). MANGANIN RESISTORS
(G) LEEDS & NORTHRUP TYPE HS (NO. 2284-A) MOVING COIL D.C GALVANOMETER
(H) LEEDS & NORTHRUP STUDENT TYPE SLIDE WIRE
(I) GENERAL RADIO OR LEEDS & NORTHRUP DECADE RESISTORS
(J) LEEDS & NORTHRUP NO 4205 1 Ω STANDARD RESISTOR
(K) LEEDS & NORTHRUP TYPE K POTENTIOMETER
(L) LEADS FOR MEASURING COUNTER CURRENT EMF
(M) LEADS FOR MEASURING THERMOCOUPLE EMF
(N) LEADS FOR MEASURING HEATER CURRENT
(O) COMMON MEASURING LEAD
(P) LEEDS & NORTHRUP NO. 8240 DOUBLE POLE TEN THROW ROTARY SELECTOR SWITCH
(Q) LEEDS & NORTHRUP NO. 3284 DPDT KNIFE SWITCH
(R) DECADE & SLIDE WIRE RHEOSTAT

FIG. 14. CIRCUIT DIAGRAM

and standardized by determining the voltage drop across the 1 ohm standard resistor (J). Through the other end of the switch (Q_1), the battery could be connected in series with a dummy heater resistance of 2 ohms (F_1), through which it could be caused to discharge at the expected rate for a few hours before actual use so as to allow its voltage to become stabilized.

The thermocouple leads were likewise connected through a similar switch (Q_2) in series with the galvanometer (G) and certain resistors (F_4 and F_5). These resistors were so chosen as to make the total resistance in series with the galvanometer equal to its critical damping resistance, as supplied by the manufacturer. The switch (Q_2) contained provision for connecting the galvanometer in series with a dummy manganin resistance (F_2) equal to that of the thermocouple, for test purposes.

A counter current tapped off from a balancing potentiometer in series with a 2 volt low discharge storage cell was connected as shown, in order to allow one to balance out the relatively large basic galvanometer deflection r sulting from the heating of the hot junction, and to brin the beam back so that the desired fluctuations with flo will fall on the recording scale. The voltage to be tappe from the potentiometer to accomplish this was determin for each cannula in each circuit at the time of calibration and set at the determined figure at the beginning of each experiment and left constant.

In order to compensate readily for shifts in the zero point of the galvanometer and for variable stray parasitic thermal currents arising in the circuit or cannula, a compensating potentiometer was connected, as shown, through a reversing switch (Q_4). With switches Q_1 and Q_3 open, and switch Q_2 connected to the thermocouple, extraneous thermal EMF's which might be present and causing error can be detected and nullified. It has been our practice to test rather frequently for these shifts and when necessary to use the compensating potentiometer to reset the galvanometer beam to its proper zero point while it is in

series with the thermocouple, unaffected by heater or balancing current. The batteries providing power for the balancing and for the compensating potentiometers were kept on continuous discharge.

Provision was made for conveniently and accurately measuring the voltage tapped off from the balancing potentiometer, the voltage drop across the 1 ohm standard in the heater circuit, and the actual thermocouple voltage, by means of a Leeds and Northrup Type K potentiometer, properly connected to its own battery, standard cell, and galvanometer. This measuring potentiometer (K) could be readily connected to any of the leads from any of our 3 circuits by means of the rotary selector switch (P).

The circuit was constructed with great care so as to conform in every possible respect with the standards necessary for reliable performance in critical low-voltage D.C. circuits. All permanent connections were carefully soldered, all resistors were of manganin, and all switches, lugs, connecting wires, etc., were selected so as to minimize the variations in resistance or voltages with temperature or other variables.

Calibration

The cannulae were calibrated with defibrinated beef blood, filtered through nylon bolting cloth, run by gravity pressure through the cannula, and measured in graduates. Calibration was carried out in a water bath at approximately 38°C. Variations in bath temperature within the range of body temperature fluctuations did not affect the calibration.

The heater current necessary to give desired sensitivity and the counter current necessary to bring the galvanometer beam to bear on the recording scale were determined for each thermocouple in each circuit. The sensitivity of the couple could be adjusted by varying the heating current, high sensitivity being achieved by high heating currents. A sensitivity giving a galvanometer deflection of 2 cm. for every doubling or halving of flow was found convenient for most cannulae.

In calibration, a series of various blood flows were passed through the cannula with heater and counter currents set, and deflections of the galvanometer noted. A sigmoid curve was obtained when voltage or galvanometer deflection was plotted against log of flow in cc. per second. The curves were conveniently plotted on semi-logarithmic paper, with voltage or deflection on the linear and flow on the logarithmic coordinates (Figure 15A).

In calibration, a definite zero flow deflection, stable to within a few millimeters, was obtained and found to be repeatable in the animal. In this respect, the instrument differs from those of Baldes and Herrick (21) and of Rein (20), which have no definite zero flow deflection (Figure 15D) (see Herrick, Baldes and Sedgwick (30)). Zero flow equilibrium is achieved when the heat supplied by the heating current is taken away solely by conduction through the cannula and fluid, and by convection. Zero flow cannot be charted on semi-logarithmic paper, but the deflection point was placed on the charts and assumed to be the point at which the curve becomes vertical. Galvanometer deflection decreased progressively as flow increased from zero.

Changes in counter current from the balancing or compensating potentiometers merely moved the calibration curve laterally along the voltage coordinates without distorting the curve in any way. Figure 15A shows 2 curves taken with the same heating current with the same cannula, but with different counter currents—the number of millivolts tapped from the balancing potentiometer being given for each curve. Theoretically, the 2 curves should be entirely parallel, separated from each other by the same distance along the abscissa for any given ordinate. Calibration curves departing significantly from this ideal were discarded.

The part of the curve for fast flows is very steep, and was found to be less repeatable than other portions (Figure 15B). In practice, it was usually possible by proper choice of heating current to place this steep portion beyond the range of flows met with physiologically. The entire curve below this steep portion could be used satisfactorily. Very slow flows were met with only terminally in our experiments, and we found it convenient to calibrate our cannulae with a heating current sufficiently large to give a range of galvanometer deflections from zero to maximal flow too large to be encompassed by our recording scale, which was 12 cm. wide. Accordingly we customarily made 2 curves with the same heating current but with different balancing currents (as in Figure 15A). It was possible to record almost all of the records on the high flow curve, the low flow curve being reserved for special purposes.

The calibration curves were repeatable and stable, and it was not necessary to calibrate each unit after or during each experiment. Figure 15B shows points from 6 calibration runs with straight forward flow on a single cannula, each run being taken on separate days, scattered over about a month's time. The small degree of scatter is evident. Also charted are points taken with pulsatile flow involving a phase of back flow, the amount of back flow being given for each point in terms of percentage of net forward flow. Points taken with back flow up to 40 per cent of net forward flow show no more scatter than the points taken with even forward flow. The reason for this is inherent in the structure of the thermocouples and their placement, and is discussed below. The curves were not affected by varying concentrations of red cells within limits met with in experimental animals, nor by changing the conductivity or the environment around the cannula in the bath, as by packing it in cotton, enclosing the cannula in a rubber tube, or leaving it free in the stirred fluid of the bath, as long as the temperature of the bath was the same as that of the blood used for calibration. With the heater currents used in practice, the maximal temperature of the hot junction at zero flow was 2 to 6° above blood temperature, depending on the mass of metal and plastic close to the heater, and on the heater current.

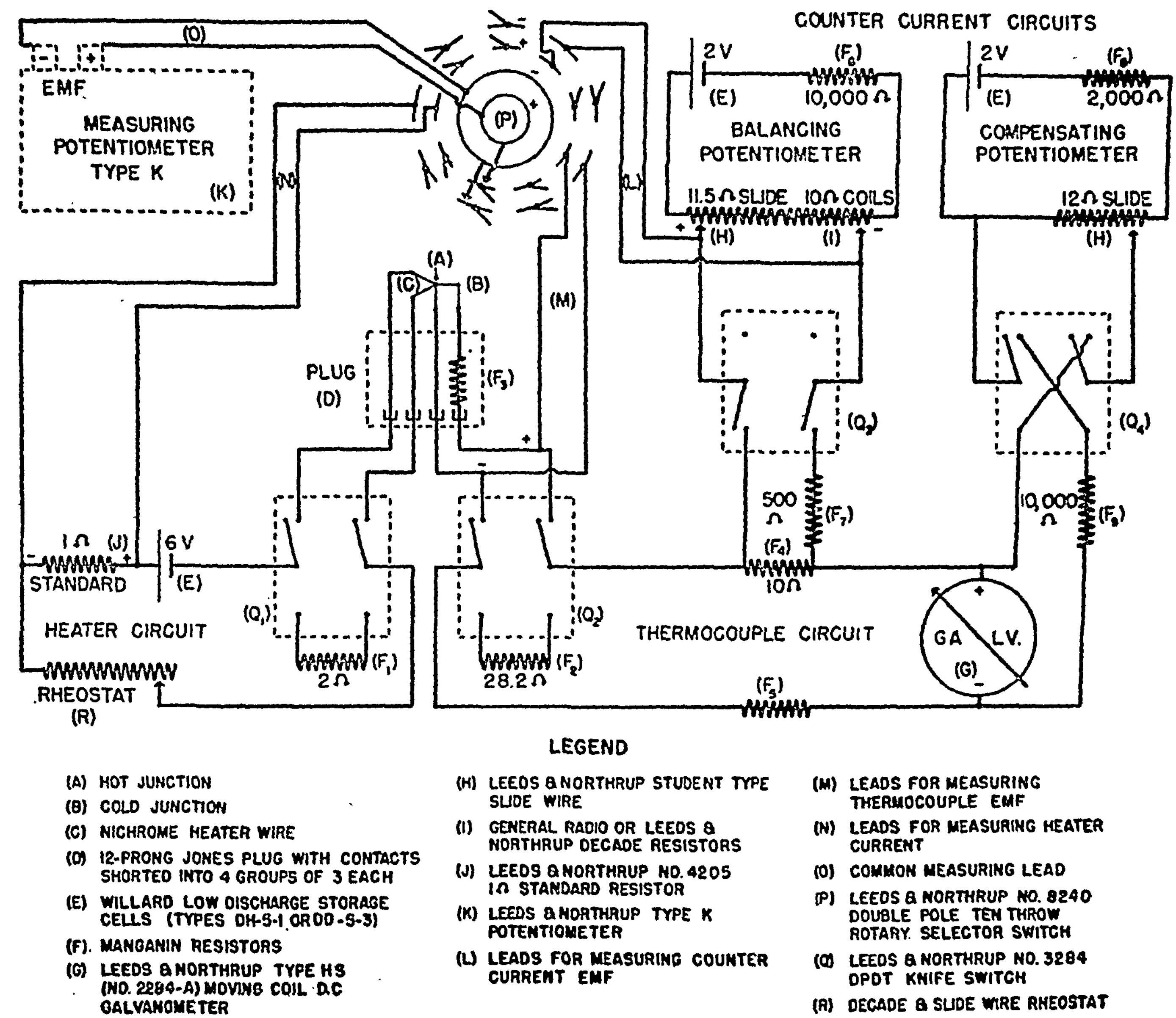


FIG. 14. CIRCUIT DIAGRAM

and standardized by determining the voltage drop across the 1 ohm standard resistor (J). Through the other end of the switch (Q_1), the battery could be connected in series with a dummy heater resistance of 2 ohms (F_1), through which it could be caused to discharge at the expected rate for a few hours before actual use so as to allow its voltage to become stabilized.

The thermocouple leads were likewise connected through a similar switch (Q_2) in series with the galvanometer (G) and certain resistors (F_4 and F_5). These resistors were so chosen as to make the total resistance in series with the galvanometer equal to its critical damping resistance, as supplied by the manufacturer. The switch (Q_2) contained provision for connecting the galvanometer in series with a dummy manganin resistance (F_2) equal to that of the thermocouple, for test purposes.

A counter current tapped off from a balancing potentiometer in series with a 2 volt low discharge storage cell was connected as shown, in order to allow one to balance out the relatively large basic galvanometer deflection resulting from the heating of the hot junction, and to bring the beam back so that the desired fluctuations with flow will fall on the recording scale. The voltage to be tapped from the potentiometer to accomplish this was determined for each cannula in each circuit at the time of calibration and set at the determined figure at the beginning of each experiment and left constant.

In order to compensate readily for shifts in the zero point of the galvanometer and for variable stray parasitic thermal currents arising in the circuit or cannula, a compensating potentiometer was connected, as shown, through a reversing switch (Q_4). With switches Q_1 and Q_3 open, and switch Q_2 connected to the thermocouple, extraneous thermal EMF's which might be present and causing error can be detected and nullified. It has been our practice to test rather frequently for these shifts and when necessary to use the compensating potentiometer to reset the galvanometer beam to its proper zero point while it is in

series with the thermocouple, unaffected by heater or balancing current. The batteries providing power for the balancing and for the compensating potentiometers were kept on continuous discharge.

Provision was made for conveniently and accurately measuring the voltage tapped off from the balancing potentiometer, the voltage drop across the 1 ohm standard in the heater circuit, and the actual thermocouple voltage, by means of a Leeds and Northrup Type K potentiometer, properly connected to its own battery, standard cell, and galvanometer. This measuring potentiometer (K) could be readily connected to any of the leads from any of our 3 circuits by means of the rotary selector switch (P).

The circuit was constructed with great care so as to conform in every possible respect with the standards necessary for reliable performance in critical low-voltage D.C. circuits. All permanent connections were carefully soldered, all resistors were of manganin, and all switches, lugs, connecting wires, etc., were selected so as to minimize the variations in resistance or voltages with temperature or other variables.

Calibration

The cannulae were calibrated with defibrinated beef blood, filtered through nylon bolting cloth, run by gravity pressure through the cannula, and measured in graduates. Calibration was carried out in a water bath at approximately 38°C. Variations in bath temperature within the range of body temperature fluctuations did not affect the calibration.

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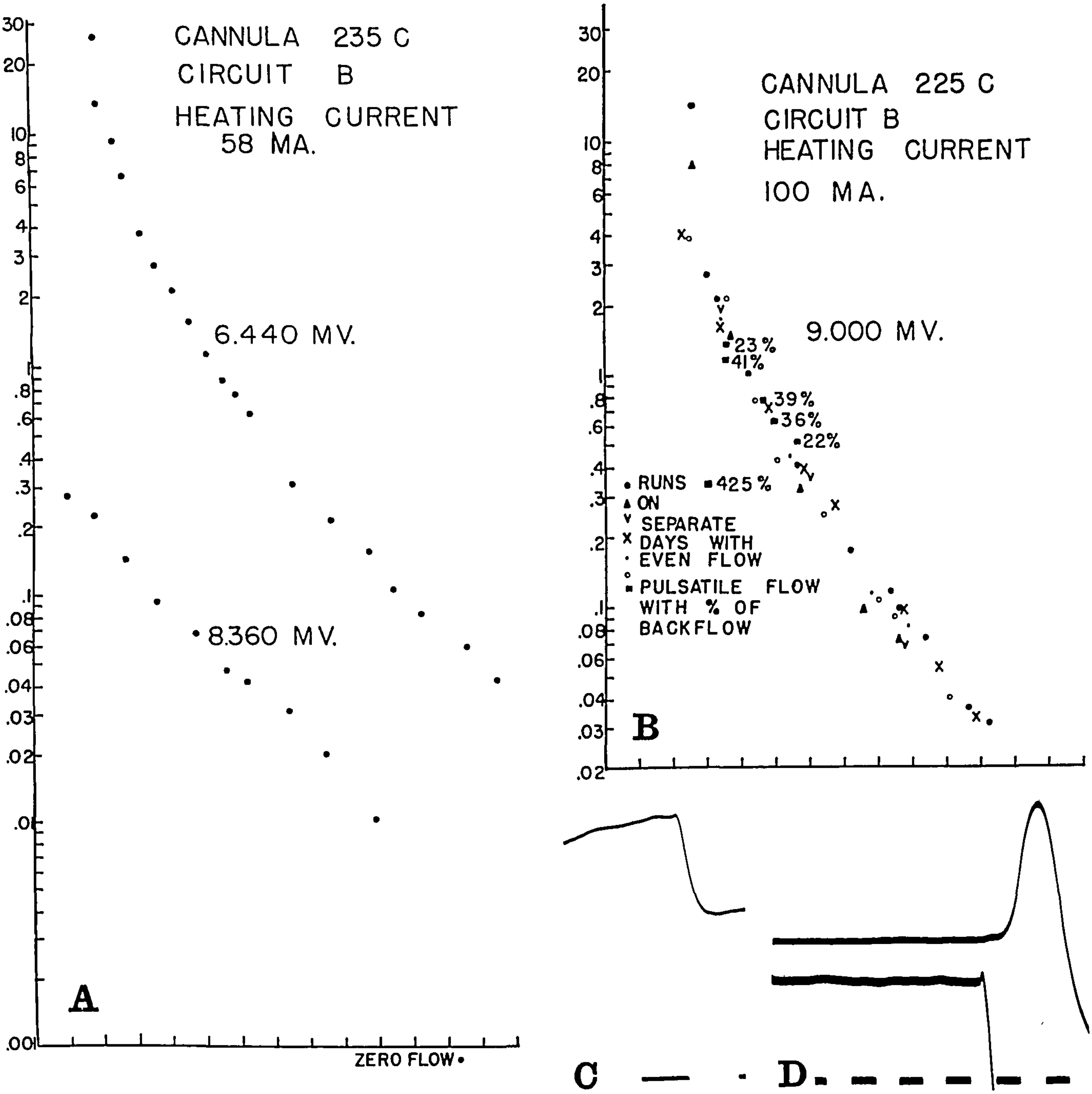

Fig. 15A. Calibration Curves of a Typical Cannula

Each curve was taken with the same heating current, but with different counter current—the voltage tapped from the balancing potentiometer being given in each case. Ordinates: Blood flow in cm.^{3} per second. Abscissae: Galvanometer deflection in cm. One mm. deflection is equivalent to about 0.5 microvolts of thermocouple voltage.

B. Calibration Points Taken with a Different Cannula, Showing the Amount of Scatter Obtained with 6 Different Runs with Straight Forward Flow and 1 Run with Phasic Flow Involving Back Flow

Each run was taken with the same heating and counter currents, but on different days, scattered over a month's time. Back flow is expressed in terms of percentage of net forward flow. Ordinates and abscissae as in Figure 15A.

C. Record of Flow Through a Cannula to Show Speed of Response

Note that the beam comes to rest within about 1.5 seconds of the time of producing a sudden change of flow through the cannula. Duration of time signal—3 seconds.

D. Comparison of Speed and Directional Reliability of Response of This Flowmeter with Baldes Thermostromuhr

The upper beam is the record of a Baldes unit in series with one of the present cannulae which is recording on the lower beam. At the spike artefact on the lower beam a sharp decrease in flow was produced, causing an immediate downward deflection of the beam recording from our instrument. The Baldes unit showed no change for a few seconds, and then gave a spurious deflection in the direction of increased flow, followed by a swing toward decreased flow. The small spike at the beginning of downswing in the lower record is an electrical artefact, induced from the coil of the signal magnet. Such effects can be avoided by shielding. Duration of time signal—3 seconds.

Time characteristics

The mounted thermocouples had variable time characteristics: those with small tips mounted in metal cannulae with little excess insulation responded more rapidly than thermocouples with tips in lucite cannulae, or with rather bulbous plastic coverings. At fast flows, the thermocouples changed temperature very rapidly with changes in flow, the limiting factor in speed of response being the time lag of the critically damped galvanometer coil, which had a period of 1.5 seconds (Figure 15C).

At changes to slow flows, or when flow stopped, thermal equilibrium was achieved more slowly, as under these conditions an appreciable amount of fluid had to be heated. Because of the high heat capacity of water, the passage of an appreciable number of seconds was required before the heater could provide enough calories to heat up the water to the point where increased temperature difference between heater and fluid gave rise to an equilibrium involving loss of heat principally by conduction and convection. Thus, at slow flows or when flow stops, the limiting factor for speed of response is the thermal lag of the fluid surrounding the heater. The actual time required for complete equilibrium to be achieved when flow was suddenly stopped varied with the nature of the cannula and insulation, but ranged, in general, between 5 and 10 seconds.

Cannulation

The insertion of the cannulae requires that the vessels be dissected, the animal heparinized, the blood vessels clamped and cut open, the cannula inserted, filled with salt solution, and secured, and the flow through the vessel reestablished. As a rule, the total time during which the flow through any vessel was shut off for cannulation was from 1 to 3 minutes. This short time was made possible largely by the use of special ligature clamps made by drilling the ends of mosquito snaps as shown in Figure 16. Before cannulation, 2 waxed braided silk ligatures were passed around the vessel and threaded through the holes in the ligature clamp, as shown. After insertion of the cannula, the ligatures were drawn tight around each bead of the cannula and clamped. This required less time than would be required to tie knots. The flow was then reestablished in the vessel, final ties were secured around the cannula, and the clamps, which thus served a temporary purpose, were then removed.

Performance and sources of error

After cannulation, the thermocouple was plugged into the circuit and the heater and counter currents set at the figures determined in calibration. The precautions taken in choice of materials and batteries and in construction of the circuit were sufficient to give adequate stability of heater and counter currents, provided the heater battery was allowed to discharge at the expected rate for a few hours before actual use. Heater current and counter current voltage were checked from time to time during each experiment, and adjustments were rarely necessary.

However, the instrument was not entirely stable and free from drift when used in animals. The bulk of the instability was traceable to the cannula and thermocouple itself. The feature of mounting the heater and thermocouple tips well inside the blood stream was adopted in order to free these elements as much as possible from temperature variations in the media surrounding the vessel. This feature has been only partially successful; apparently, there is some slight thermal conduction through the cannula wall and along the wires and insulation to the thermocouple junctions, for the deflection of the galvanometer could be influenced somewhat by changes in the temperature of the environment of the cannula. The influence of these variables was reduced by packing cotton around the cannula where it rested in the animal, by closing the wound snugly over it, and by covering the general area with cotton or blankets so as to keep the temperature as constant as possible.

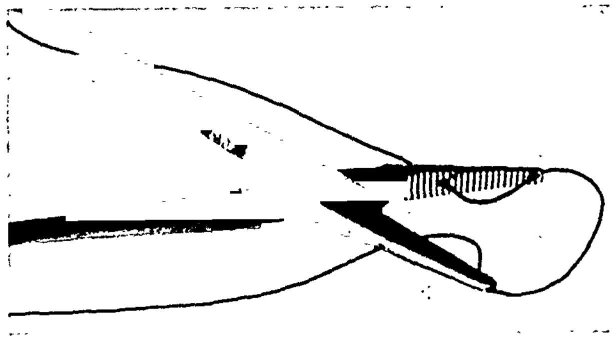

Fig. 16. Threaded Ligature Clamp Made by Drilling Mosquito Snap

Even these precautions did not suffice to eliminate drift entirely, and it was necessary to check for, and correct electrically, currents arising from varying ambient temperature by use of the compensating potentiometer and switches Q_1 and Q_3, as described under "*Circuit.*" By correcting for these factors as frequently as the nature of the experiment demanded, it was possible to get flow records in which the amount of drift was known or corrected, with consequent improvement in accuracy. Each episode of checking involved disconnecting the thermocouple from heat and counter current, with a consequent swing of the galvanometer beam to a resting place near its zero point off the recording scale. After checking and, if necessary, resetting the zero point, closure of switches Q_1 and Q_3 brought the beam back to its proper recording position, leaving a short gap in the flow recording line corresponding to the time the galvanometer beam was off the scale. If resetting of the compensating potentiometer was necessary, a shift appeared in the projection of the reconstituted flow meter record. The degree of instability and drift ordinarily encountered, as revealed by this shift, can be seen in the flow records (Figures 6, 9). Quite possibly, error from varying environmental temperature might be further reduced by refinements in the placement and construction of the thermocouple tips. However, we feel other sources of inaccuracy are so large as not to justify any great experimentation along such lines.

A further source of error arose from the fact that all portions of a moving blood stream may not be at exactly the same temperature. As shown by Franklin and McLachlin (31), blood from various tributary streams emptying into a larger vein may remain as separate and distinct "stream lines" for a considerable distance down the larger vein. Such a stream from a tributary vein draining surface tissues may be at a temperature different from a similar stream from deep tissues, and these 2 streams may varyingly influence the 2 junctions of the couple. Changes in the relative proportions of these components might produce galvanometer deflections which would not represent true flow changes.

This factor was troublesome at times in flow measurements in peripheral veins, such as the jugular and femoral. It has not been so troublesome in deep veins, such as the portal or mesenteric, or in arteries. It is not always possible to correct for this error by means of the compensating potentiometer, as inflow of cold blood from a surface vein, for example, may vary phasically with respiration, or may be changed sufficiently by pharmacological agents or other factors to make interpretation of the records extremely difficult.

Error can arise within the cannula if it be angulated with respect to the blood vessel leading into it, so that the rapid portion of the blood stream deflects against the hot junction, giving rise to an apparent flow recording which exceeds the true one. Conversely, if the stream is deflected against the wall of the cannula opposite the hot junction, the hot junction may find itself in a component of the stream moving more slowly than normally and record a falsely low flow. In practice, it has usually been possible to align the cannula so that this deflection error was small or absent. At times, however, especially in deep cavities, such good alignment has been difficult to maintain, and erroneous records have resulted. Error from angulation may be considerable in short, wide cannulae; it is negligible in cannulae which are long in relation to their diameter.

A further source of error arises from the fact that the rate at which heat is taken away from a heated wire by a moving stream of fluid does not bear a linear relation to velocity, whereas the EMF from a thermocouple and the resistance changes in a wire are very nearly linear functions of temperature changes within the limits of the application to blood flow measurements. Hence, the mean deflection obtained by a mechanical integration, by the galvanometer, of the thermal currents varying with phasic flow changes will not represent true mean flows over the corresponding period of time. The size of this deviation would vary with the nature and period of the phasic changes, but the test runs with the phasic flow in the calibration bath (Figure 15B) suggest that the error may be more of theoretical than of practical importance, as far as this instrument is concerned.

Gregg *et al.* (22) and Shipley *et al.* (23) pointed out that back flow during certain phases of diastole could cause great error in the Baldes thermostromuhr. Back flow in our flowmeter was of less consequence as a source of error than in the Baldes instrument since (*1*) the cold junction was mounted in a position where it was not affected by warmed blood washed back from the heater, and (*2*) heat was applied directly to the hot junction so that blood washed back on it still retained, initially at least, some heat from its recent forward passage past the couple, and hence cooled it less in back flow. As stated previously, phasic back flow up to 40 per cent of the net forward flow was tolerated by our instrument without appreciable error.

General appraisal and comparison with other thermal methods

This instrument was sufficiently stable to give calibration points with a scatter of about ± 10 per cent or ± 15 per cent above or below the mean flow in favorable portions of the curve (Figure 15B). This was better than we were able to achieve using comparable precautions with the Baldes apparatus (cf. Gregg *et al.* (22)).

However, in the animal, the instrument was less reliable, and the experimental data now available do not allow us to set any rigid limits as to its accuracy. Obviously, in the presence of angulation, or of marked difference in temperature between various components of the blood stream flowing through the cannula, or in the presence of fibrin clots, it may be totally inaccurate.

Under reasonably satisfactory operating conditions, when external temperature was not fluctuating too rapidly, when angulation was absent, and when the blood within the vessel was at even temperature, fairly consistent results were obtained, since (*1*) flow values in a given vessel, corrected for body surface of the animal, compared favorably with flows similarly taken and corrected from other animals under comparable conditions, and (*2*) flow measured in an artery was close to flow in the corresponding artery on the other side, or with flow in the corresponding vein. Good checks between artery and corresponding vein would appear to render unlikely any error from phasic changes or from back flow, which would scarcely be expected to affect both artery and vein alike or to the same degree. Examples of mean values of flows (with standard errors) in various vessels, obtained during a considerable series of experiments, are given in Table VII. These mean values are in fair agreement with corresponding figures obtained by other workers with other methods, but the fact that occasional flows were recorded well above or below the mean suggests that technical errors may at times be considerable. Similar discrepancies in comparing flows in corresponding vessels in the same animal were also sometimes encountered.

Our instrument utilized refinements in circuit construction beyond those used by Rein (20), Baldes and Herrick (21), Gibbs (27), and Schmidt and Walker (28). These refinements have been effective in improving the time characteristics, stability, and accuracy of the instrument. Attaching the thermocouple directly to the heater, as done by Gibbs (27) and Schmidt and Walker (28), gave a stable zero flow deflection, which was lacking from the instruments of Baldes and Rein, as pointed out by Herrick, Baldes, and Sedgwick (30). The influence of environmental changes in temperature, which contribute most

markedly to the inaccuracy of the Baldes thermostromuhr (Gregg *et al.* (22); Shipley *et al.* (23)) and which would likewise affect the instrument of Rein (20) and of Schmidt and Walker (28) was greatly reduced in our instrument by mounting the thermocouples directly inside the blood stream. In addition, provision was made for nullifying error from this source when it was present. Errors in the Gibbs (27) method, arising (*1*) from changes in the caliber of the vessel, and (*2*) from possible shift of the needle tip from rapid axial portions of the blood stream to slower circumferential portions, were eliminated in the present instrument by mounting the thermocouples in a rigid cannula in a fixed and permanent position relative to the moving blood stream (in the absence of angulation). By reducing the amount of inert material around the heater and thermocouple, the time characteristics have been improved so that the limiting factor as to speed of response at fast flow changes is the mechanical inertia of the galvanometer, and at slow flow, the thermal lag of the surrounding fluid.

This considerable lag of response with changes to very slow flows or to stopped flows—the lag being a consequence of the high heat capacity of water—appears to be a hitherto unappreciated limitation of thermal flow recording in fluids. Machella (26) apparently allowed himself to be led into error from failure to consider this factor. He used a small heated nickel wire which would change temperature rapidly, and which would follow fast flow changes quite closely. His wire operated at about 6° above blood temperature. When flow stopped, it was necessary for this wire to heat the surrounding blood to nearly this temperature before equilibrium could be achieved, and this would require an interval longer than the diastolic period. Hence, the instrument was inadequate for detecting stoppage of flow in the aorta in diastole, and his denial of this stoppage is not warranted. Shipley, Gregg, and Schroeder (8), who obtained evidence of diastolic stoppage or even back flow in the aorta using another method, were unable to explain the discrepancy between their results and those of Machella. The factors mentioned here would provide a possible explanation for this discrepancy.

Basic limitation of thermal methods

Because of the numerous attempts to develop instruments to record blood flow by thermal methods, with results never wholly satisfactory, it would seem advisable to list factors which limit the usefulness and accuracy of all thermal methods. Some of these factors have been pointed out before with respect to the particular instrument of Baldes and Herrick, by Gregg *et al.* (22) and Shipley *et al.* (23).

1. Thermal methods cannot be relied upon to record rapid phasic changes in flow in fluids involving phases of very slow flow or of complete stoppage. The thermal lag of fluid surrounding the heated element is such that several seconds may be required for equilibrium to be attained after flow stops.

2. Uneven temperature in various portions of a given blood stream in a vessel may be present to a degree sufficient to cause temperature changes in the heated element, falsely representing flow changes of considerable magnitude.

3. The temperature of the heated element does not bear a linear relationship to flow. Hence, when pulsatile flow changes are present, an integration of voltage or resistance changes by a recording system will not represent a true mean flow.

4. Fluid moving in either direction cools a heated element equally well, and hence, phasic back flow will record as forward flow.

These limitations, when combined with the great difficulty encountered in attempting to free completely the heated element from varying environmental factors, appear to be sufficient to render unlikely the development of a very satisfactory blood flow recording system utilizing the thermal principle.

Summary

A heated thermocouple flowmeter suitable for providing automatic and continuous recording of blood flow in cannulated vessels of anesthetized heparinized animals is described. The instrument is designed to eliminate or minimize certain factors producing error in previous thermal methods of flow recording. The advantages and limitations of the instrument are discussed and its performance is compared with that of other thermal flowmeters.

Certain basic limitations and sources of error inherent in all thermal methods of flow recording appear to be sufficient to render unlikely the development of a very satisfactory blood flow recording system utilizing the thermal principle.

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COMPARATIVE VALUE OF BROMSULPHALEIN, SERUM PHOSPHATASE, PROTHROMBIN TIME, AND INTRAVENOUS GALACTOSE TOLERANCE TESTS IN DETECTING HEPATIC DAMAGE PRODUCED BY CARBON TETRACHLORIDE

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Although tests of liver function have been studied for many years, it is only recently that any intensive comparative studies have been made (1 to 3). Liver function studies are performed (*a*) to detect hepatic damage, (*b*) to aid in prognosis and assist in determining any surgical risk, (*c*) to differentiate obstructive from non-obstructive jaundice. The detection of hepatic damage is complicated by the large number of functional activities performed by the liver. As various functions of the liver will be affected to different degrees by a given type of damage, the results of a liver function test will depend upon which function is tested. Thus, while a given test is positive, others may be negative. This has led to the general concept of a *dissociation* of liver functions and liver function tests.

Although tests of hepatic function are used on experimental animals, no experimental comparison has been made of the newer liver function tests. In this investigation, 5 liver function tests were studied in dogs receiving small doses of carbon tetrachloride, twice a week, to ascertain (*a*) the comparative sensitivity of the tests in detecting hepatic damage, (*b*) the constancy, or change in value, of a test as the doses of carbon tetrachloride were continued, (*c*) whether or not *association* or *dissociation* of the liver function tests was present.

METHODS

Adult dogs, weighing 8.4 to 14.6 kgm., were used as experimental animals. Gardner *et al.* (4) have extensively studied the pathological changes produced in the liver of dogs by carbon tetrachloride, and from their data, a dose of 0.5 cc. of carbon tetrachloride per kgm. of body weight was selected. The carbon tetrachloride was mixed with an equal volume of corn oil and administered by stomach tube before feeding. The dogs generally received this dose twice a week (Tables I and II) and the liver function tests were performed at various intervals to ascertain which tests would first detect any hepatic damage. The bromsulphalein method of Rosenthal and White (5) was used, except that 5 mgm. of dye per kgm. of body weight was injected in place of the original 2 mgm. dose. A single blood sample was withdrawn after 30 minutes and the concentration of dye determined, using the standards developed for the 2 mgm. dose (4 mgm. dye per 100 cc. dilute NaOH equivalent to 100 per cent), as it is more difficult to determine the amount of dye with a higher concentration of standards. Thus, with the 5 mgm. dose and the original set of standards, the bromsulphalein retention will range above 100 per cent during liver damage. Normal dogs generally retain 2 to 12 per cent of bromsulphalein at the end of 30 minutes. Any retention of dye above 15 per cent at the end of 30 minutes is regarded as definitely abnormal. The dye concentration was determined in a block comparator, using a blue glass filter. Serum phosphatase was determined according to the method of Bodansky (6). A value above 5 units per 100 cc. of serum is considered abnormal. The one-stage technic of Ziffrin *et al.* (7) was used to determine prothrombin time, and the result compared with control dogs is expressed as per cent of normal. This method was used rather than the more sensitive two-stage technic as the simplicity of the test lends itself to "bedside" usage, making it of interest to compare its sensitivity with the other "laboratory" tests. In the initial part of the study, thromboplastin was prepared from rabbit lung, but later a commercial preparation was used.[2] A prothrombin time of 85 per cent or less was considered definitely abnormal, and values between 86 and 90 per cent, as questionably abnormal As the oral glucose and galactose tolerance tests have proven inadequate, the intravenous galactose tolerance test of Bassett, Althausen, and Coltrin was used (8). After a control blood sample was taken, 1.0 cc. of 50 per cent galactose per kgm. of body weight was injected intravenously, and blood samples taken at 30, 60, and 75 minutes. In initial studies a sample was also taken at 90 minutes, but this did not add any further information and was discontinued. Glucose was removed from the

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[2] Thromboplastin, generously supplied by the Abbott Laboratories.

blood sample by fermentation according to Raymond and Blanco's method (8). Non-fermentable reducing substances were determined in the filtrate by the method of Folin and Wu. The amount of reducing substance in a fasting sample of blood was subtracted, and because of a difference in reducing power, a correction value of 24 per cent was added to the final result. The direct and indirect Van den Bergh tests were performed according to standard procedures.

Five of the dogs were fed the laboratory stock diet consisting of meat scraps, bones, bread, yeast, and cod liver oil. For comparison, and to make the results more reproducible, the second group of 5 dogs was fed a modified form of Cowgill's synthetic casein diet No. III (9), plus a daily supplement of yeast. The results did not indicate any difference with respect to the diet used. All liver function tests were performed at least 16 hours after the previous feeding.

RESULTS

The control determinations and the effect of carbon tetrachloride on the various liver function tests are shown in Tables I and II.

Bromsulphalein retention. This test was the most sensitive of the ones studied. Seven dogs (Nos. 2, 3, 6, 7, 8, 9, 10) developed an abnormal retention of bromsulphalein after 2 doses of carbon tetrachloride were administered. Dogs Nos. 1 and 5 showed an abnormal retention of dye after 3 doses of carbon tetrachloride. The re-

TABLE I

Ability of various liver function tests to detect hepatic damage in dogs receiving carbon tetrachloride

A. Dogs fed stock diet

Days of experiment	Weight	Bromsulphalein retention	Serum phosphatase	Prothrombin time	I. V. galactose tolerance			Comment
					30′	60′	75′	
Dog No. 1								
	kgm.	*per cent*	*units per 100 cc.*	*per cent normal*	*mgm. per 100 cc.*			
	11.3		2.17	100	47	19	6	Control
	11.4	8	3.52	98	58		7	Control
1								CCl_4 admin.
4								CCl_4 admin.
7	11.0	5	2.86	103				
8								CCl_4 admin.
9			6.43					
10	11.4	50	5.99	78	44	13	6	
11								CCl_4 admin.
15								CCl_4 admin.
19								CCl_4 admin.
21	11.2	130	6.23	88	84	55	32	
24				88				CCl_4 admin.
27								CCl_4 admin.
29	11.1	220	9.33	63	75	31	14	
31								CCl_4 admin.
34								CCl_4 admin.
36	10.9	200	10.67	60	78	37	17	
Dog No. 2								
	8.4	5	3.45		37	5	0	Control
	8.2	2	4.58	98	52	16	2	Control
1								CCl_4 admin.
5								CCl_4 admin.
7	10.6		15.91					
8	10.4	125	15.57	94	49	9	5	
9								CCl_4 admin.
13								CCl_4 admin.
17								CCl_4 admin.
19	10.1	140	14.17	90	55	22	5	
22								CCl_4 admin.
25								CCl_4 admin.
27	9.5	250	28.47	94	46	8	5	
29								CCl_4 admin.
32								CCl_4 admin.
34	9.4	230	24.43	90	64	22	8	

TABLE I—*Continued*

Days of experiment	Weight	Bromsulphalein retention	Serum phosphatase	Prothrombin time	I. V. galactose tolerance			Comment
					30′	60′	75′	
Dog. No. 3								
	kgm.	*per cent*	*units per 100 cc.*	*per cent normal*	*mgm. per 100 cc.*			
	10.0	12	1.01	98				Control
	9.5		1.87	107	38	16	7	Control
1								CCl_4 admin.
4								CCl_4 admin.
6	10.0	55	4.03	90	42	22	11	
12								CCl_4 admin.
17								CCl_4 admin.
18	10.5	200	7.31	72	64	38	29	CCl_4 admin.
24								CCl_4 admin.
25				54				
27								CCl_4 admin., died 2 days later in convulsions
Dog. No. 4								
	14.6	5	2.43					Control
	14.8	5	1.10	100	57	17	3	Control
1								CCl_4 admin.
4								CCl_4 admin.
7	14.3	17	0.45		48	14	4	
8								CCl_4 admin.
10	14.8	15	1.19	108				
12								CCl_4 admin.
14	14.5	50	5.25	89	55	32	11	
18								CCl_4 admin.
20	13.6	90	3.01	95				
26								CCl_4 admin.
31								CCl_4 admin.
32	14.5	125	7.33		70	41	24	
38								CCl_4 admin.
39	14.4		11.57	89				
Dog. No. 5								
	9.8	8	3.52	97				Control
	10.1	10	3.18		34	15	2	Control
1								CCl_4 admin.
4								CCl_4 admin.
6	10.2	10	4.18	95	28	12	2	
7								CCl_4 admin.
9	10.0	30	8.28	102	38	18	4	
10								CCl_4 admin.
14								CCl_4 admin.
16	9.8	80	10.06	90	26	11	3	
20								CCl_4 admin.
24								CCl_4 admin.
26	9.6			98	40	16	5	
27								CCl_4 admin.
31								CCl_4 admin.
33	9.9	175	12.84	93	30	15	4	

sults on dog No. 4 were less definite, a slight dye retention being obtained after two doses of CCl_4, only to return to 15 per cent (the upper limit of normal in our series) on the next test, and becoming definitely abnormal after 4 doses of CCl_4. None of the other tests became abnormal before the bromsulphalein test. The retention of bromsulphalein progressively increased as further doses of CCl_4 were administered.

Serum phosphatase. Six dogs (Nos. 1, 2, 5, 6, 8, 9) showed an increase in serum phosphatase above normal at the same time the bromsul-

phalein retention became abnormal. Dogs Nos. 3, 7, and 10 developed a rise in serum phosphatase slightly after the bromsulphalein test indicated hepatic damage, following another dose of CCl_4. In these 9 dogs, the rise in serum phosphatase showed a close correlation with a retention of bromsulphalein. In dog No. 4, however, the rise in serum phosphatase definitely occurred later than the retention of bromsulphalein. The prothrombin time or intravenous galactose tolerance test did not detect any hepatic damage earlier than the serum phosphatase test.

Prothrombin time. The prothrombin time, as determined by the one-stage technic, detected hepatic damage in only one dog (No. 1) as early as did the bromsulphalein test. In the other dogs, the prothrombin time became abnormal after the bromsulphalein and serum phosphatase tests. Four of the dogs (Nos. 2, 4, 5, 6) did not develop an abnormal prothrombin time during the course of the experiment.

Intravenous galactose tolerance. In some cases, this test was difficult to interpret, but, in general, the intravenous galactose tolerance test detected liver damage after the prothrombin time. Four dogs (Nos. 5, 7, 8, 10) did not show any abnormal response. The control values for the intravenous galactose tolerance test varied from dog to dog, and could not be standardized as closely as the bromsulphalein retention, serum phosphatase, or prothrombin time.

The *indirect Van den Bergh* test was performed on 4 dogs, but as it did not show any change from normal during the experiment, it was discontinued.

TABLE II

Ability of various liver function tests to detect hepatic damage in dogs receiving carbon tetrachloride

B. Dogs fed synthetic diet

Days of experiment	Weight	Brom-sulphalein retention	Serum phosphatase	Prothrombin time	I. V. galactose tolerance			Comment
					30′	60′	75′	
Dog No. 6								
	kgm.	*per cent*	*units per 100 cc.*	*per cent normal*	*mgm. per 100 cc.*			
	11.3	8	2.67		58	17	8	Control
	11.4	5	3.67	98	48	13	5	Control
1								CCl_4 admin.
4								CCl_4 admin.
7	11.4		14.54					
8	11.4	90	14.40	100	56	17	3	CCl_4 admin.
11	11.3			100	68	23	15	
12								CCl_4 admin.
14	11.3	110	16.78		40	24	14	
15								CCl_4 admin.
18								CCl_4 admin.
20	11.4	175	13.10	87				
26								CCl_4 admin.
31								CCl_4 admin.
32	11.8		11.53		74	32	18	
34	11.6	175	12.35	90				
Dog. No. 7								
	14.1	10	4.50	95	25	10	8	Control
1								CCl_4 admin.
5								CCl_4 admin.
8	13.6	35	4.70	97	27	9	7	CCl_4 admin.
12	13.6		6.80	89	21	8	6	CCl_4 admin.
14	12.7	175	7.42	50	18	10	8	
15								CCl_4 admin.
19								CCl_4 admin.
21	11.6	200	11.32	67		10	1	
23								CCl_4 admin.
26								CCl_4 admin.
28	11.8	250	11.43	67	10	7	4	

TABLE II—*Continued*

Days of experiment	Weight	Brom-sulphalein retention	Serum phosphatase	Prothrombin time	I. V. galactose tolerance			Comment
					30′	60′	75′	
				Dog. No. 8				
	kgm.	*per cent*	*units per 100 cc.*	*per cent normal*	*mgm. per 100 cc.*			
	11.3	12	4.50					Control
	11.4	10	4.99	98	26	16	4	Control
1								CCl_4 admin.
5								CCl_4 admin.
7	11.1	35	5.62	102	28	11	2	
8								CCl_4 admin.
11	11.2	35	5.16	95	24	11	3	
12								CCl_4 admin.
14	11.4	125	5.01	94	20	6	4	
15								CCl_4 admin.
19								CCl_4 admin.
21	11.6	65	6.50	70	17	11	5	
23								CCl_4 admin.
26								CCl_4 admin.
28	11.4	70	7.14	74	20	4	1	
				Dog No. 9				
	10.0	15		104				Control
	10.1	10	2.81	92	53	14	4	Control
1								CCl_4 admin.
4								CCl_4 admin.
5	10.0	25	6.59	90	62	12	7	
7								CCl_4 admin.
9	9.8	50	8.24	85	56	22	8	
10								CCl_4 admin.
14								CCl_4 admin.
16	9.7	100	7.48	71	78	32	15	
17								CCl_4 admin.
21	9.9	80	10.81	78				
24								CCl_4 admin.
26	9.7	130	14.2	59				
28								CCl_4 admin.
32								CCl_4 admin.
34	9.6	200	20.3	50	82	28	12	
				Dog No. 10				
	12.8	12	2.84	98	42	18	7	Control
1								CCl_4 admin.
5								CCl_4 admin.
7	12.6	40	3.46	92	32	16	5	
8								CCl_4 admin.
10	12.9	90	8.02	105	39	15	6	
11								CCl_4 admin.
13	13.1			82	49	20	9	
14								CCl_4 admin.
18								CCl_4 admin.
20	12.6	150	12.54	72	36	18	4	
22								CCl_4 admin.
27								CCl_4 admin.
29	13.0				40	12	6	
31								CCl_4 admin.
33	12.6	175	18.38	66	47	17	8	

DISCUSSION

Of the liver function tests studied, the brom-sulphalein retention (5 mgm. dose) and serum phosphatase tests were found to be the most sensitive in detecting hepatic damage produced by administering carbon tetrachloride. Next in order of sensitivity was the prothrombin time, as determined by the one-stage technic. The

TABLE III

Summary of liver function tests, comparing the number of doses of carbon tetrachloride after which a given test became abnormal

Dog No.	1	2	3	4	5	6	7	8	9	10
Bromsulphalein retention	3	2	2	2,4	3	2	2	2	2	2
Serum phosphatase	3	2	4	4,7	3	2	3	2	2	3
Prothrombin time	3	—	4	—	—	—	4	6	3	4
I. V. galactose tolerance	6	9?	4	4	—	3	—	—	5	—
Indirect Van den Bergh	—	—				—	—			

least sensitive tests were the intravenous galactose tolerance and serum bilirubin tests. A further comparison of these tests is shown in Table III.

It was to be expected that the determination of serum bilirubin in dogs would be less sensitive than the other tests, as injected bilirubin is excreted faster in the dog than in man (10). The serum of normal dogs does not contain bilirubin. If the doses of CCl_4 were continued longer, serum bilirubin would eventually be present (11).

Although the serum phosphatase test was practically as sensitive as the bromsulphalein test, the rise in serum phosphatase was greater in some dogs than in others, this being especially true of dog No. 8. This difference in the rise in serum phosphatase of dogs receiving CCl_4 has previously been observed (11). Although the serum phosphatase rises above normal, the degree of change does not always parallel the increase in bromsulphalein retention. It has been found that bile fistula dogs develop an abnormal retention of bromsulphalein and a rise in serum phosphatase (12). In the bile fistula dogs, as in the dogs receiving CCl_4, the degree of rise in serum phosphatase also varied. Bile phosphatase was measured in the bile fistula dogs, and it was found that dogs showing a high serum phosphatase excreted *more* bile phosphatase than normal. When the rise in serum phosphatase was not as great, it was found that only a small amount of bile phosphatase was present. It is possible that a similar underlying change in phosphatase metabolism will account for the difference in the rise of serum phosphatase of dogs receiving CCl_4.

Although serum phosphatase has been known to rise in various types of hepatic damage, the rise during obstructive jaundice is higher, and most clinical studies have been directed to using the test in differentiating the two types of jaundice. However, the values during hepatic and obstructive jaundice overlap sufficiently to make the test of doubtful value in differential diagnosis. Experimentally, serum phosphatase has shown a close correlation with the dye retention tests during hepatic damage produced (*a*) by feeding dogs a protein-free diet (13), (*b*) during experimental hyperthyroidism (14), (*c*) in bile fistula dogs (12), and (*d*) in the present study with CCl_4. No extensive clinical reports have been made on the serum phosphatase test compared with other sensitive liver function tests. The experimental results suggest a possible clinical value of this test, which is relatively simple to perform, requiring only one blood sample. Bone diseases, however, must be ruled out.

If the diet is excessively high in carbohydrate or a non-fasting blood sample is used, the serum phosphatase will be increased (15,16). In the present study, however, all blood samples were taken at least 16 hours after the previous feeding.

The prothrombin time, determined by the one-stage technic, was not found to be as sensitive as the bromsulphalein or serum phosphatase tests. In an early report, Ziffren *et al.* (7) obtained good correlation between the one-stage and two-stage technic for prothrombin time in all except one patient with hepatic disease. In a later study, however, they observed that the one-stage technic was satisfactory for following response to vitamin K therapy, but was not found to be as sensitive in detecting liver damage as the two-stage technic, for it measures both the rate of conversion of prothrombin and the concentration of prothrombin (17 to 20). The conversion rate of prothrombin is faster in the dog than in man, although little difference is found in the number of prothrombin units in the plasma (20). Thus, any increase in the rate of conversion of prothrombin may compensate for a deficiency of prothrombin. The one-stage technic will, however, give a practical index of any tendency to bleed.

The liver damage produced can be considered fairly acute and does not entirely correspond with conditions that occur in attempting to assess liver function in human beings. Also, it is not always possible to transfer the results of animal experiments to human beings. This is especially true with studies on jaundice, as dogs do not

develop jaundice to any degree, except with severe intrahepatic damage or complete biliary obstruction. Thus, in the dog, it would be difficult to evaluate accurately the diagnostic importance of various liver function tests in the differential diagnosis of jaundice. It should also be born in mind that the rate of conversion of prothrombin is faster in the dog than in man, which might make this test less sensitive in the dog. Keeping these differences in mind, the dog should still prove to be a valuable test animal in studies of hepatic function.

The liver is known to perform a large number of functional activities. Clinical comparison of liver function tests have shown that in a given case some tests are positive while others may be negative. These positive tests are said to be more sensitive than those giving a negative response. This in turn led to emphasis being placed on a *dissociation* of the various liver functions. The word dissociation generally is taken to mean that the various functions of the liver are separated, or dissociated, so that injury to the liver may interfere with some hepatic functions without affecting others. However, the fact that some tests are negative while others are positive does not furnish adequate proof for the dissociation of liver functions. On the other hand, it would be more remarkable if the various hepatic functions were affected to an equal degree during liver damage. It is more reasonable to suppose that whether one or more tests will detect liver damage will depend on the degree of liver damage. This idea is borne out by the experimental evidence in this paper. The bromsulphalein test detected hepatic damage first, but as further doses of CCl_4 were administered, other liver function tests in turn became abnormal. Thus, it would seem that rather than a dissociation of liver functions, these various hepatic functions are correlated, or *associated*, so that as the degree of damage increases, more of the functions of the liver will become abnormal. Hence, emphasis should not be placed, as it has in the past, on the dissociation of liver functions, but rather on the most sensitive, reliable, test for detecting hepatic damage. This "sensitive" test, first detecting hepatic damage alone, would be followed by other tests becoming abnormal as the degree of damage increases. This idea of liver function tests emphasizes the quantitative *association* of these tests rather than a qualitative *dissociation* of liver function tests.

SUMMARY

1. Ten dogs received 0.5 cc. of carbon tetrachloride per kgm. of body weight, twice a week by stomach tube. The number of doses of carbon tetrachloride required to produce an abnormal liver function was fairly constant. Seven dogs developed an abnormal liver function, which became progressively more abnormal as the administration of CCl_4 was continued, after 2 doses; 2 dogs, after 3 doses; and 1 dog, after 4 doses of carbon tetrachloride.

2. Of the liver function tests studied, the bromsulphalein retention (5 mgm. dose) was the most sensitive in detecting the type of damage produced.

3. The serum phosphatase value rose above normal in all except 1 dog at the same time as, or shortly after, the bromsulphalein retention became abnormal. This test was practically as sensitive as the dye retention test used.

4. The prothrombin time (one-stage technic) was not as sensitive as the serum phosphatase or bromsulphalein tests, but still detected hepatic damage before the intravenous galactose tolerance test. In some dogs, the prothrombin time and galactose test remained normal throughout the period of carbon tetrachloride administration. No change in serum bilirubin was found, when studied in 4 of the dogs.

5. Emphasis is placed on an *association* of hepatic functions and liver function tests, more of which will become abnormal as the degree of damage increases, rather than on a dissociation of liver function tests.

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FACTORS IN THE RESISTANCE OF GONORRHEA TO SULFONAMIDES

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INTRODUCTION

The problem of resistance to sulfonamide therapy[1] in male gonorrhea is well known. Cox (1) states that, in varying degrees, clinical resistance is found in approximately 25 per cent of cases treated with sulfadiazine or sulfathiazole. More recently, the incidence of resistance has risen to approximately 50 per cent of cases admitted to the Boston Dispensary (2). This resistance is manifested either by persistence of symptoms or by persistence of positive cultures in asymptomatic carriers. Since gonorrhea is usually a self-limited disease, Cox emphasizes the importance of considering all cases as drug failures which do not clear up within 2 weeks after starting sulfonamide therapy. It is likewise important to recognize relapse after apparent cure as a manifestation of resistance, which is often missed in the clinic unless patients are followed every few days by smear, culture, and symptomatology for at least 4 weeks.

Petro (3) summarizes the factors in sulfonamide resistance, emphasizing (*1*) factors interfering with the transport of drug to the site of infection in adequate dosage, (*2*) factors interfering with proper drainage of the products of inflammation, (*3*) factors within the invading organism, and (*4*) factors within the host and its bodily defenses.

Determination of factors within the gonococcus responsible for this clinical resistance is a logical initial approach. To this end, correlation of the clinical course with laboratory observations on the growth characteristics of the organisms isolated from individual cases in the presence of sulfonamides has been reported by Cohn *et al.* (4), Bang and Bang (5), Petro (3), and, most recently, by Lankford *et al.* (6), in a series of 200 female cases. All of these investigators report that organisms from clinically resistant cases show, by their various methods, an increased tolerance to sulfonamides in the laboratory. Harkness (7) reports a correlation in the great majority of cases, but emphasizes his belief that some failures are due to an increased tolerance of the host to the drug, irrespective of the organism. In these few cases, the organism is in fact responsive to sulfonamides *in vitro*, and he reports successful clinical response after switching to another sulfonamide to which the host presumably has not acquired a tolerance. (See discussion: practical application.)

In the work reported here, our aim has been:

(*1*) To confirm the work mentioned above by an independent method.

(*2*) To provide the experimental basis for a rapid method of identifying sulfonamide-resistant strains with sufficient accuracy to permit prediction of the possible success of sulfonamide therapy in any given case.

The authors wish to express, at this point, their appreciation of the invaluable advice and guidance offered throughout this work by Dr. J. Howard Mueller of the Department of Bacteriology of Harvard Medical School, and by Dr. Oscar F. Cox, director of the G. U. clinic of the Boston dispensary, where the cases were studied.

EXPERIMENTAL METHOD

Pure cultures of gonococcus were isolated from male patients and incubated at partial tension on casein-hydrolysate-starch agar slants at 37° C. (8).

Promptly at 18 hours, standard suspensions of the growths were made in buffered saline,[2] pH 7.4, to match

[1] Sulfanilamide and sulfapyridine are no longer commonly used in the therapy of gonorrhea; in this paper, "sulfonamide therapy" refers to the use of sulfathiazole, sulfadiazine, or sulfamerizine therapy—these being the newer and more effective drugs.

[2] Saline was used for this purpose because it was felt that greater accuracy could be obtained in preparing the standard suspensions than by the use of broth or serum. Because of the toxic effect of the saline, it is important to inoculate the plates immediately after the dilutions of the

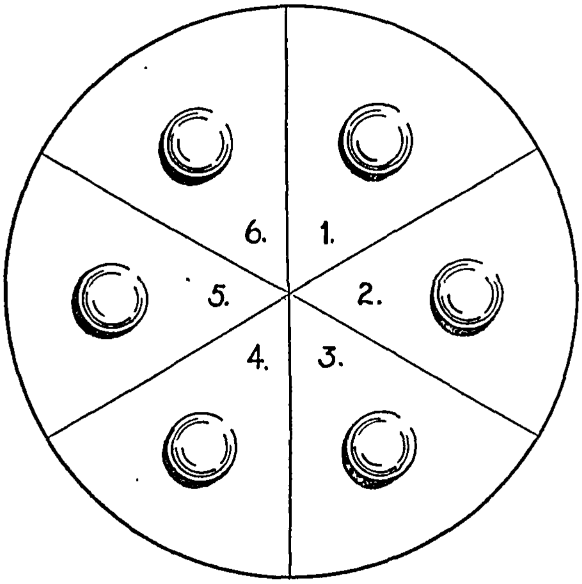

FIG. 1. SKETCH OF INOCULATED PETRI PLATE

a McFarland suspension tube No. 1 under fluorescent lighting. Serial tenfold dilutions of 10^{-1} to 10^{-5} were then made in similar saline.

A drop of each of the 6 suspensions (standard and 5 dilutions) was placed in turn, each upon a marked sector of a starch agar petri plate,[3] divided into 6 parts. Eight other plates were similarly inoculated, each of these plates containing varying amounts of sulfonamides as follows:

Plate No. 1 contained no drug,—*Control*
Plate No. 2 contained sulfathiazole, 0.001 per cent
Plate No. 3 contained sulfathiazole, 0.003 per cent
Plate No. 4 contained sulfathiazole, 0.005 per cent
Plate No. 5 contained sulfathiazole, 0.010 per cent
Plate No. 6 contained sulfadiazine, 0.001 per cent
Plate No. 7 contained sulfadiazine, 0.003 per cent
Plate No. 8 contained sulfadiazine, 0.005 per cent
Plate No. 9 contained sulfadiazine, 0.010 per cent

The drops of inoculum, from a capillary pipette of approximately constant caliber, were merely *placed* upon the medium, as shown in Figure 1, rather than streaked. By so doing, a constant number of organisms covered an approximately constant area of medium.

The plates were then incubated rightside up to avoid running of the drops, and examined at 18 hours. Growth appeared as a solid circle within the discrete boundaries of the original drops of inoculum, as discrete colonies within the same region, or finally as a faint ring visible only by reflected light, indicating that some slight reproduction had occurred in the first few hours of incubation before bacteriostasis was complete. In sectors reported as negative on each plate, not even a ring was visible where the drop of inoculum had been placed, the agar appearing completely untouched.

The control plate showed an average of 50 colonies in the inoculum from the sixth dilution of organisms (conc. 10^{-5}). Since such quantitation of the number of organisms in each suspension was easily repeatable by the method of standardization used, it was possible to express the results of sulfonamide inhibition *in vitro* as the product of two factors, both essential in estimating resistance to the drugs: (*1*) The concentration of drug in the medium, and (*2*) the concentration of viable organisms inoculated.

In vitro results were reported in two ways (Tables I and II, Figure 2):

I. *Resistance index.* This arbitrary figure is the sum of all the positive growth readings found on the 4 plates containing sulfadiazine or sulfathiazole. The value of 1 was given to each clearly visible drop of growth, while drops visible only by reflected light were given a value of 0.5. By inspection of Figure 1, it will be apparent that this

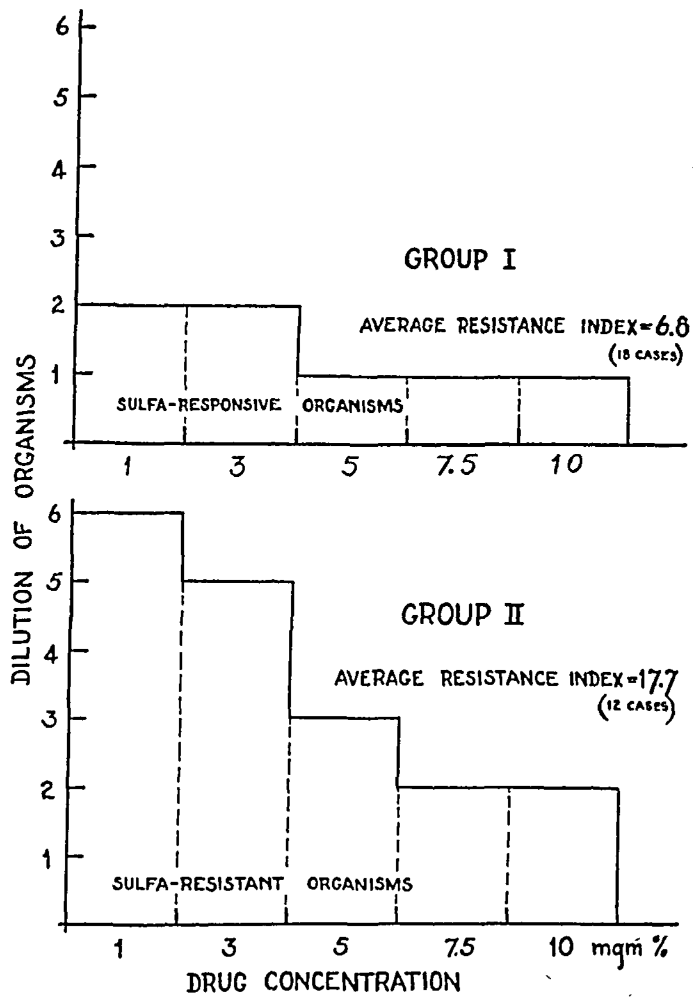

FIG. 2. GROWTH OF GONOCOCCI ON SULFATHIAZOLE-CONTAINING MEDIA

original suspension have been made. Careful buffering to pH of 7.4, checked by a potentiometer each time before using, kept this toxic effect down to a minimum.

[3] The p-amino benzoic acid, usually added routinely to promote growth of gonococci isolated from patients receiving sulfonamides, was omitted from the test medium. It might also be mentioned that the success of the method depends in part upon the use of a highly reproducible medium such as Mueller and Hinton's starch agar, containing a minimum of p-aminobenzoic acid, as well as relatively constant amounts of growth-promoting and growth-inhibiting substances.

TABLE I

Individual case results

Case number	Clinical history: Number of previous Gc. infections	Clinical history: Previous sulfonamide therapy	Drug used*	Days after onset therapy started	Cure in: Days	Cure in: Visits	*In vit* Resistance index: S.T.	*In vit* Resistance index: S.D.	*In vit* Maximum dilution: S.T.	*In vit* Maximum dilution: S.D.	Remarks
1	2	0	S.D.	4	0 to 3	1	6	5	2	1.5	Complete cures after first visit
2	?	0	S.D.	1	0 to 2	1	7	5	1.5	1.5	
3	2	?	S.M.	?	0 to 5	1	5	5	1.5	1.5	
4	2	0	S.M.	15	0 to 2	1	6	6	1	2	
5	0	0	S.M.	5	0 to 6	1	5	6	1.5	2	
6	0	0	S.D.	30+	0 to 1	1	7	6	2.5	1.5	
7	0	0	S.D.	7	0 to 1	1	7	7	2.5	2	
8	1	0	S.T.	0	0 to 2	1	6	7	1.5	2	
9	2	0	S.D.	4	1 to 5	2**	7.5	7	2.5	2	
10	2	0	S.M.	6	0 to 2	1	5	8	1.5	2	
11	0	0	S.M.	2	1 to 3	2**	8	8	2.5	3.5	
12	1	0	S.D.	3	0 to 2	1	9	9	2.5	2.5	
13	0	0	S.T.	1	0 to 2	1	7	9	2	3	
14	0	S.A.	S.D.	23	0 to 2	1	8	9	2.5	2.5	
15	2	0	S.D.	?	1 to 2	2**	8	9	2.5	3	
16	0	0	S.M.	1	0 to 2	1	8	10	2.5	3	
17	1	0	S.T.	2	0 to 1	1	6	10	1.5	3	
18	1	0	S.M.	18	0 to 2	1	7	10	2.5	3	
19	?	0	S.T.	12	5 to 6	4	12	14	3.5	5.5	Intermediate
20	1	0	S.D.	4	6 to 8	4	16	16	4.5	4	
21	0	S.T., S.A.	Local	?	90+	6+	17	16	5	6	Complete sulfonamide failures
22	0	0	S.M.	11	26	6+	16	17	6	6	
23	2	S.T.	Local	?	90+	6+	15	17	6	6	
24	1	0	S.D.	2	50+	6+	18	19	6	6	
25	0	?	S.T., S.D.	10	30+	6+	16	21	6	5	
26	1	0	S.D., S.T.	1	37	6+	18	22	6	6	
27	2	0	S.M.	0	38	6+	21	22	6	6	
28	0	0	S.D.	?	35	6+	13	23	5	6	
29	0	0	S.T., S.D.	0	150+	6+	22	24	6	6	
30	1	Yes (?)	S.D.	5	?	—	19	24	6	6	
31	1	0	S.D.	3	30+	6+	16	24	6	6	
32	0	Yes (?)	Local	?	30+	6+	15	24	6	6	

* SA = sulfanilamide, SD = sulfadiazine, SM = sulfamerizine, ST = sulfathiazole.

** The first of these 2 visits was less than 24 hours after starting therapy, at which time all showed negative smears, only slight discharge, and positive culture only in case # 11.

sum of positive readings expresses the ability of the organism to grow in the presence of a wide range of sulfonamide concentrations in all dilutions of the organism itself, thus the two vital factors in estimating sulfonamide resistance, mentioned above, have been accounted for by this value.

II. *Maximum dilution.* At 0.001 per cent of sulfadiazine, the sharpest differences between strains were observed, as measured by the maximum dilution of organisms at which growth occurred. This figure was therefore recorded to show how one single plate with this concentration of sulfadiazine could be used alone to test the resistance of strains under a simplified, more practical method (9).

EXPERIMENTAL RESULTS

Thirty-two cases of proven male gonorrhea were studied as outlined above. They fell into two main groups and one small intermediate group (of 2 cases), both on the basis of clinical

TABLE II

Summary of the average in vitro *results*

	Resistance Index	Maximum Dilution
Group I—Responsive		
Sulfathiazole *in vitro*	6.8	2
Sulfadiazine *in vitro*	7.6	2
Group II—Resistant		
Sulfathiazole *in vitro*	17.7	5.4
Sulfadiazine *in vitro*	21	5.7
Group III—Intermediate*		
Sulfathiazole *in vitro*	14	4
Sulfadiazine *in vitro*	15	4.7

* Data from this group are insufficient for any definite conclusions.

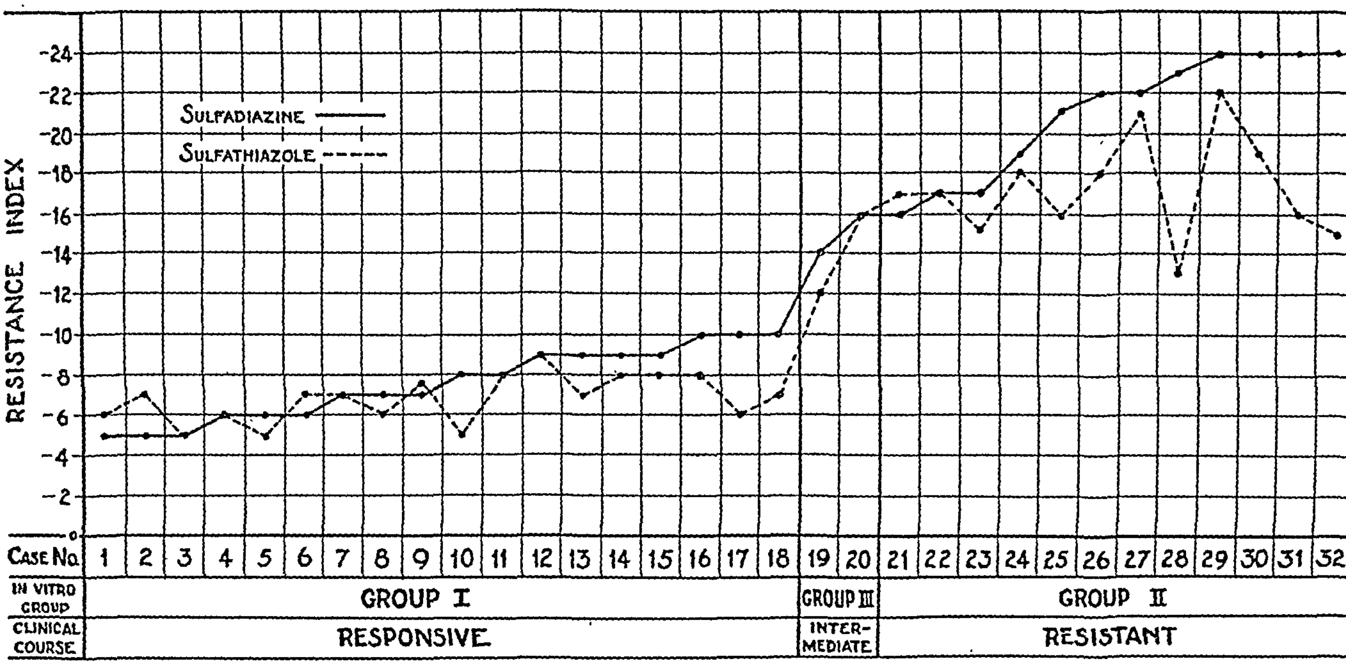

FIG. 3. CLINICAL AND *In Vitro* RESISTANCE OF 32 CASES

response and the *in vitro* test. A strikingly close correlation was obtained in every case (See Table I and Figure 3).

Group I, consisting of 18 patients, showed negative smears and cultures, and were asymptomatic on the first examination after therapy was started, remaining negative at each subsequent follow-up examination. This first examination was usually made 24 to 48 hours after sulfonamide was initially administered, as shown in Table I, but in some cases, the first visit was made 3, 5, or 6 days after the beginning of treatment. All of the strains isolated from this group showed *Resistance Indices of 5 to 9* with sulfathiazole and *5 to 10* with sulfadiazine. They *grew out at most to only the third dilution* of organisms inoculated on the plates containing the lowest concentration of each drug, 1 mgm. per cent.

Group II, consisting of 12 patients, continued to show evidence of infection for 26 days or more; in other words, by Cox's criteria, they were true sulfonamide failures. Four of these cases had cultures taken for study before any sulfonamides were given, 4 were previous drug failures before admission to the clinic, and 4 were cultured at varying times after unsuccessful sulfonamide therapy in the dispensary. All of these 12 cases showed *Resistance Indices* varying from *13 to 24*, and all grew out to a *Maximum Dilution of 5 to 6*, at a concentration of 1 mgm. per cent of each drug.

Group III consisted of 2 cases, who were not cured after the first visit or first 48 hours of therapy, but who subsequently became completely negative, soon enough to attribute the cure to sulfonamides. One case was cured in 6 to 8 days, the other in 5 to 6. *In vitro*, the 2 strains also gave results intermediate between the 2 groups, I and II, with *Resistance Indices of 12 to 16*, and *Maximum Dilutions of 4 to 5.5*. This group is so small that one can only speculate on the presence of an intermediate mildly sulfonamide-resistant strain.

DISCUSSION

I. Theoretical

The results reported above confirm the correlation, noted by others, between the clinical course and the *in vitro* tolerance of the infecting gonococcus to sulfonamides. Such a complete correlation, with no exceptions found, constitutes positive evidence that sulfonamide resistance in male gonorrhea depends upon factors within the organism rather than upon factors within the infected host.

Additional evidence against any appreciable contribution to sulfonamide resistance by host factors follows, some of which throws light upon

difficulties encountered in sulfonamide therapy of this disease:

(*1*) The uniformly short courses of the responsive group of cases (less than 2 days in all cases followed daily) are hard to explain except on a basis of absolute bacteriostasis by the drug alone. Host factors such as immune body titre could be regarded as important in determining the reaction of the patient to sulfonamide therapy only if marked variations in the length of these courses were seen. It is also well known that gonococci prevented from reproducing will be destroyed rapidly by "autolysis," or by a rapid aging process. This may explain the rapid action of sulfonamides in responsive cases without aid from phagocytes and immune bodies.

(*2*) The rare intermediate type of clinical course showed positive signs of disease for about a week, followed by complete sulfonamide cure within 14 days. Even these cases, however, were infected with strains which showed a partial *in vitro* sulfonamide resistance compatible with the clinical picture and were best explained by variations within the organism rather than within the host.

Lankford *et al.* (6) have found that, under certain conditions, varying degrees of sulfonamide resistance, each of which can be estimated quantitatively, could be induced *in vitro* from strains previously found responsive to sulfathiazole.[4] Such changes in the ability of the organism to tolerate the sulfonamides resembled in general the process of mutation, and the degree of resistance, once attained, remained constant. It is not unreasonable to suggest that a similar process may occur in nature, with varying degrees of sulfonamide resistance, perhaps acquired by mutation, accounting for several distinct clinical courses under sulfonamide therapy. In our small series, at least 2 variant strains, the immediately responsive and the completely resistant types, and possibly a third intermediate variant show up both clinically and *in vitro*. It is possible that other mutants with further variations in sulfonamide resistance also exist. It must be remembered, however, that as yet no demonstration has been made of a responsive strain becoming resistant *in vivo* under the influence of sulfonamide therapy in gonorrhea. Such a phenomenon *has* been demonstrated in cases of streptococcus viridans (12), pneumococcus endocarditis (13), and pneumococcus pneumonia (14).

(*3*) Regardless of the stage of the disease at which treatment is begun, sulfonamides seem to act promptly or not at all, leaving the course of a resistant infection unchanged as far as we can tell. This fact is obvious from Table I in the column marked: "Days after onset when therapy was started." Case 14, for instance, received sulfanilamide for the first 2 weeks without effect and was first admitted to the clinic after 23 days of urethral discharge. He was found to have a positive culture, but to be infected with a strain susceptible *in vitro* to the more effective sulfonamides. His culture and symptoms became permanently negative within 24 hours of the institution of sulfadiazine therapy.

In other words, it appears that a responsive case will be cured by sulfonamides promptly, no matter how long symptoms have been present, while a resistant case will run its course, no matter how soon or how late the drug is started, or how many courses of sulfonamides are given, in the overwhelming majority of cases.[5]

[4] Landy *et al.* (10) and Stokinger, Charles, and Carpenter (11) have also induced sulfonamide resistance *in vitro* in formerly responsive strains. They identified an increase in the production of p-amino benzoic acid by the organisms with the acquisition of resistance, and offer this as a partial tentative explanation of the changes in metabolism involved.

[5] The rarity of success of a second course of sulfonamides where a first course has failed, as well as the dangers of inducing drug resistance in the host by insufficient dosage, are discussed by Cox (15), who above all would discourage the indiscriminate use of multiple courses of drug in resistant infections, especially if the organism proves to be drug resistant *in vitro* as well.

The rare prompt response actually attributable to a subsequent course of chemotherapy in an initially resistant infection, as studied in the Boston Dispensary, usually occurs in already waning infections; these cases usually have persisted for over 4 weeks, with subsidence of acute symptoms, and signs that the natural process of self-limitation of the disease is already underway. Adequate *in vitro* studies on such cases are not complete yet, but we believe that one of several circumstances may account for the success of a second course of sulfonamides where a first has failed:

(*1*) Attenuation of the organism may occur in the presence of an increasingly hostile and immune host. Such

The clinical picture of the resistant infections is therefore essentially the same as the disease seen before the days of sulfonamide therapy—in our series, infections lasting from 26 days to many months. In these cases, excluding the rare partially resistant infections, there is no evidence that sulfonamides have any appreciable therapeutic effect, by the criteria of Cox (1). *Within* this resistant group it is therefore logical that host factors may well help to determine whether a man will be free from disease within a few weeks or within a matter of months.

The essential fact remains, however, that whether or not the *initial and prompt cure with sulfonamides* will be obtained at all seems *to depend upon a contest between organism and drug in a relatively neutral host.*

II. Practical application

In all 32 cases studied, it was possible to predict the clinical course that would follow treatment with sulfonamides. It was found that a "typing" of the organisms into responsive and resistant groups could be obtained with accuracy by the use of one plate containing 1 mgm. per cent of each sulfonamide, indicating that considerable simplification of the method would be possible. (The details of a simplified method have already been worked out and published, and it is now being used routinely in the G. U. clinic of the Boston Dispensary under the direction of Dr. Cox (9).)

Recent work (2) has shown that infections may show marked variations in response depending upon whether sulfathiazole, sulfadiazine, or sulfamerizine is used. These cases are not common, but are brought more forcibly to one's attention by the fact that the variations appear correspondingly *in vitro*, as illustrated by the following case:

B. D. No. 427038 entered the clinic with a fresh untreated infection. The organism cultured before starting chemotherapy was typed *in vitro* as resistant to sulfamerizine but responsive to sulfathiazole. Meanwhile, through misunderstanding, sulfamerizine was administered to the patient, without response. After 16 days of persisting resistant infection, a course of sulfathiazole was started, with prompt and permanent response. Both the *resistance to sulfamerizine and the response to sulfathiazole* were thus predicted by the original *in vitro* typing on admission.

It is therefore wholly logical that cases should occasionally appear to be completely resistant to therapy with one drug and later respond perfectly to a second course using another drug which, in this particular infection, happens to be more effective, both clinically and *in vitro.* Harkness (7), on the other hand, believes that variations in response to different sulfonamides are due to variations in the host's tolerance for the drug. Although his explanation may also hold true in a small additional percentage, variations in potency of the several sulfonamides seem to explain adequately those cases studied in the Boston Dispensary.

The ability to predict the results of sulfonamide therapy by the use of this typing method has several obvious advantages:

(*1*) If the organism is responsive *in vitro*, the patient can be discharged safely after a short course of sulfathiazole, sulfadiazine, or sulfamerizine, without risk of relapse, and can remain ambulatory throughout treatment. *The most effective of the available drugs indicated should also be determined and administered accordingly, by typing the organism on plates containing each of the drugs available.*[6]

(*2*) The finding of an organism resistant to a sulfonamide *in vitro* will immediately indicate that the patient will probably not respond well,

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a change might appear as a decrease in the resistance to sulfonamides of the organism subsequently cultured from the patient. As yet, however, there are no reports of a resistant strain changing to a responsive variant in the host.

(*2*) A different drug may have been used for the late course, the potency of which was greater against the particular organism involved than that of the original drug. (See discussion, Practical application.)

(*3*) Host immunity may have arisen to the point at which slight aid from previously ineffective sulfonamides will complete the elimination of the infection.

(*4*) Insufficient dosage may have been used initially.

[6] In most cases, sulfathiazole, sulfadiazine, and sulfamerizine seem to be equally effective (or ineffective) in gonorrhea, both *in vitro* and *in vivo.* In the borderline cases, where differences in potency show up, sulfamerizine is definitely weaker, and sulfadiazine very slightly weaker, than sulfathiazole. Inexplicable exceptions to this order of potency occasionally appear. Sulfapyridine is of consistently lower potency, while sulfanilamide is comparatively ineffective.

if at all, to that sulfonamide. If the organism is resistant *in vitro* to all available sulfonamides, then another form of therapy is advisable. Where facilities are available, such patients might often be cured promptly by the use of fever therapy or penicillin (16, 17), both of which are effective in a high percentage of sulfonamide-resistant cases. Also, repeated courses of sulfonamides should *not* be used in these cases where chances of success are slim, and where chances for toxic effects and sensitivity formation become increasingly great.

(*3*) Finally, once a patient has failed to respond, as in Case 14, the chances of success with a second course of the same or a different drug can be determined by retyping the organisms grown out on subsequent cultures.

SUMMARY

(*1*) Thirty-two cases of male gonorrhea, treated with sulfonamides, have been analyzed.

(*2*) Correlation between clinical and *in vitro* resistance of the infecting strain to sulfathiazole and sulfadiazine has been demonstrated in every case studied.

(*3*) This correlation indicates that those factors within the gonococcus which determine *in vitro* resistance to sulfonamides also determine whether the infection will respond promptly and permanently to sulfonamides, or show varying degrees of clinical resistance.

(*4*) Host factors appear to be relatively unimportant, except in those infections in which the course is initially unaffected by drug therapy.

(*5*) The *in vitro* resistance of the organism, and hence the clinical response to be expected under sulfonamide therapy, can be accurately estimated by the method outlined.

(*6*) The clinical value of a simplified modification of this "sulfonamide resistance typing" technique has been presented. It is suggested that such *in vitro* methods have a possible application in other bacterial infections, placing the use of bacteriostatic drugs on a more rational basis, as well as discouraging the use of futile indescriminate courses of sulfonamides where the organism is obviously resistant.

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IMMUNOLOGIC STUDIES IN INSULIN RESISTANCE

I. REPORT OF A CASE EXHIBITING VARIATIONS IN RESISTANCE AND ALLERGY TO INSULIN

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Extreme resistance to insulin has been reported in a small number of diabetic patients who, for varying periods, have tolerated ten to fifty times as much insulin as that commonly required for the control of diabetes. Even with these very large doses, control of the diabetic state has not always been achieved. The subject of resistance to insulin has been reviewed recently (1). The high tolerance exhibited by these patients has been tenatively ascribed to lack of some factor necessary for the action of insulin, to endocrine imbalance or abnormality, to allergy, or to a neutralizing antibody. Previous reports contained evidence for the presence of a neutralizing factor in the blood of an insulin-resistant patient, which appeared to exhibit specificity and which was thought to be an antibody for insulin, distinct from the allergic antibody (2, 3). The course of this patient has now been followed for about 15 months and studies made during this period lend considerable support to the view that the resistance in this patient was immunologic in nature.

CASE REPORT

Patient A. M. is a 50-year-old white married female. At the age of 19, she underwent a pelvic operation following a pregnancy after which there was complete cessation of menstruation. She had been well otherwise until 1931 when she developed an appendiceal abscess which was drained surgically at the Boston City Hospital. During this admission, a diagnosis of diabetes mellitus was made and treatment with insulin was begun. No adverse reactions occurred and the patient stated that her urine became free of sugar. On discharge, she was advised to follow a diet and to take no insulin. In 1939, she was seen in the Out-Patient Department of the Boston City Hospital and treatment with protamine zinc insulin (Lilly) was begun because of glycosuria and acetonuria. About one week after resuming treatment, there were marked local reactions to the injected insulin and, at times, there were also generalized urticaria and a constricting feeling in the chest. Intracutaneous tests with beef, pork, and lamb as well as crystalline insulins indicated allergy to all these and the injections were discontinued (4).

In August 1941, she was admitted to the Boston City Hospital complaining of aching and paresthesias of both legs and feet of 2 months' duration. There had been also loss of appetite, episodes of nausea and vomiting, weakness, and weight loss. Physical examination revealed obesity, muscle tenderness of the legs, and absent knee and ankle jerks. The blood pressure was 140/80. Laboratory studies showed a three plus reaction for sugar and acetone in the urine. Albumin and many white cells were also present. Culture of the urine yielded *E. coli*. The blood hemoglobin was 78 per cent and the white count was 8,400. The differential count showed no abnormality and there was no eosinophilia. Other findings were as follows: Hinton negative; NPN 34; FBS 215; CO_2 combining power 38 per cent; chlorides 101 m.eq. A diagnosis of diabetic acidosis was made and the patient was given infusions of saline. Sulfadiazine was given by mouth for the urinary infection.

The patient warned the house officer that she was allergic to insulin. A dose of 4 units (0.1 ml. of U 40) of regular insulin was given intracutaneously on August 5, 1941. This was quickly followed by severe generalized urticaria, difficulty in breathing, and a fall in blood pressure with loss of consciousness. Adrenalin given intravenously and intramuscularly gave relief. Two days later desensitization was begun with an initial dose of 0.001 unit of crystalline insulin, subcutaneously. After 3 days, during which increasing amounts of insulin were given, 8 units were well tolerated in a single dose. Thereafter, larger doses were given until 11 days after the first dose of insulin she could tolerate 300 units in a day and 2 days later she received 570 units in a day. These amounts of insulin were given without having any observable effect on the diabetic state. No fall in the blood sugar followed the intravenous injection of 30 units of crystalline insulin. Finally, on September 2, 1941, 26 days after desensitization was begun, 860 units of regular insulin were given slowly by continuous intravenous infusion, over a period of 6 hours. Blood sugar determinations during and after this period showed no fall, but on the contrary, a gradual rise from 250 mgm. per 100 ml. to 364 mgm. per 100 ml. The injection was finally stopped because of severe urticaria, nausea, and vomiting. The total dose of insulin given during this admission was approximately 2,500 units. No further attempts were made to treat the patient with insulin at this time. X-rays of the skull showed no ab-

normalities of the sella turcica. The patient was discharged on the 33rd hospital day with instructions to follow a diet and to take no insulin.

During the following 4 months, she had fairly frequent headaches, some aching and burning of the legs and feet, nocturia one to three times, and considerable drowsiness.

In February 1942, she was admitted to the Evans Memorial Hospital for further study. The findings on physical examination were the same as those noted above with the addition of diabetic retinitis. There was no ketonuria, but the urine constantly contained large amounts of sugar and there were many white cells and variable amounts of albumin. Culture of the urine yielded *E. coli.* X-rays of the skull were negative. Endermal injection of 0.05 ml. of U 40 crystalline insulin diluted 1 : 100 (0.02 units) produced local whealing, erythema, and itching, associated with transitory mild generalized itching. Desensitization was begun on February 18, 1942 with increasing doses of insulin. Several attacks of generalized urticaria and constriction in the chest prevented rapid increases in the dosage. No insulin effect was noted until the third day (February 20) when she complained of dizziness after receiving 102 units, subcutaneously, over a period of 8 hours. The urine became free of sugar and the blood sugar fell to 84 mgm. per 100 ml. During the next 2 days, the patient received 24 and 44 units, respectively. These amounts were sufficient to clear the urine of sugar but the fasting blood sugar remained elevated. No systemic allergic reactions occurred, and redness, swelling, and itching at the site of the injections of insulin were absent or minimal. At this time, it appeared that the patient had lost her resistance to insulin and no further difficulty in the control of the diabetes was anticipated.

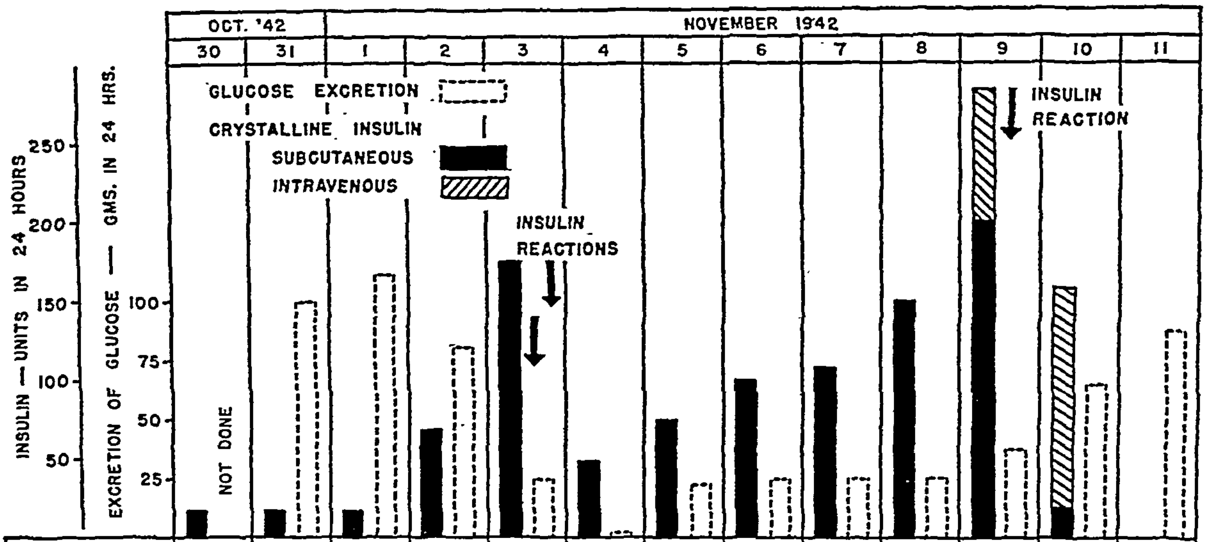
During the following 5 days, various tests were done with insulin and because of these, control of the diabetes was not attempted. When insulin therapy was again started for control of the diabetes, it was found that the patient had become resistant. On the eleventh and twelfth days after desensitization was begun, a total of 458 units of insulin was given subcutaneously without having any apparent effect on the diabetic state. Thus it appeared that the patient had again become resistant. Systemic manifestations of allergy did not occur, but local itching and redness were marked.

On March 10, 21 days after desensitization was started, 30 units of crystalline insulin were given intravenously without causing a fall in blood sugar. In a similar test with human insulin on March 11, the blood sugar fell from 360 mgm. per 100 ml. to 84 mgm. per 100 ml. in 90 minutes, indicating that the patient was not resistant to this insulin. These tests have been reported previously (2). No allergic reaction followed the injection of crystalline insulin but the test with human insulin caused severe urticaria, constriction in the chest, and nausea and vomiting. Treatment with insulin was then abandoned, the patient having received a total of approximately 1,500 units in 23 days. Sulfathiazole was given for the urinary infection and thiamin chloride for the peripheral neuritis. She was discharged on the 36th hospital day with instructions to follow a diet.

She remained well until May 1942 when she developed scarlet fever for which she was hospitalized. During this period, the fasting blood sugar was consistently high and the urine was strongly positive for sugar. She made an uneventful recovery and received no insulin during the illness. For the following 4 months, she felt fairly well but developed increasingly severe symptoms of peripheral neuritis during the 5th month.

She was again admitted to the Evans Memorial Hospital in October 1942 having had no insulin for 8 months. The physical findings were unchanged. Intracutaneous tests with beef, pork, and crystalline insulin, and with a preparation of human insulin to be described below, were strongly positive. Control of the diabetes was again attempted and the sequence of events was very similar to that of the admission in the spring of 1942. The patient's course for the first 12 days of insulin therapy is shown in Figure 1. The high degree of allergy again prevented the administration of effective amounts of insulin during the first day. An attempt to desensitize the patient by giving daily injections of 10 units in divided doses during the first 3 days was unsuccessful. Fifty-six units given in the course of 6 hours on the fourth day caused severe urticaria and tightness in the chest. On the fifth day, November 3, 1942, 140 units given subcutaneously over a period of 8 hours were tolerated. This amount caused a hypoglycemic reaction, the blood sugar falling to 82 mgm. per 100 ml. A second reaction occurred when 30 units more were given in divided doses. Thus, for the second time, relatively small amounts of crystalline insulin were effective. On the sixth day (November 4), the fasting blood sugar was 98 mgm. per 100 ml., and only 46 units of insulin were required to prevent the excretion of sugar in the urine. It should be noted, however, that the small amount of insulin required may have been due in part to the low caloric intake on that day. A temporary decrease in the intensity of the local reactions at the site of the injections was again noted.

From the seventh to the tenth day, the daily dose of insulin was steadily increased. The fasting blood sugars remained elevated and some sugar was excreted in the urine. This suggested that resistance to insulin was returning. On the eleventh day (November 9), after 200 units had been given subcutaneously and the urine continued to show a strong reduction, 82 units were given intravenously in divided doses over a period of 3 hours. This caused a hypoglycemic reaction, the blood sugar fell to 67 mgm. per 100 ml. and the urine became free of sugar. On the twelfth day, 20 units given subcutaneously followed by the intravenous injection of 140 units given over a period of 7 hours caused a decrease in the amount of sugar in the urine temporarily but no hypoglycemic reaction occurred. No insulin was given on the thirteenth day and the total excretion of glucose rose to 83 grams, indicating that the increasing daily doses of insulin from the seventh to the twelfth day were having some effect on the diabetes.



		30	31	1	2	3	4	5	6	7	8	9	10	11
FBS		425			395		98		286		425	285	203	
DIET	CARBOHYD.	127	195	180	122	256	90	180	150	150	150	150	150	150
	PROTEIN	73	59	75	53	73	28	74	60	60	60	60	60	60
	FAT	75	63	113	52	72	50	72	60	60	60	60	60	60
	CALORIES	1475	1583	2037	1168	1964	922	1664	1400	1400	1400	1400	1400	1400
I.N.A.*		0					0		0			+	+	

* INSULIN NEUTRALIZING ACTIVITY

FIG. 1. RESPONSE TO TREATMENT AFTER 7 MONTHS WITHOUT INSULIN

On the 15th day (November 13) after desensitization was begun, an intravenous insulin tolerance test with 30 units of crystalline insulin showed a rise followed by a slight fall in the blood sugar (Figure 2). The patient was then desensitized with a preparation of human insulin and, on the following day, an intravenous insulin tolerance test with human insulin was done.

The human insulin used in this test was prepared during the summer of 1942. Assay was made by comparing its capacity to lower the blood sugar in rabbits with that of a known sample of commercial (Lilly) insulin. The glucose determinations were made in the fasting state and then at 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 5 hours after the injection of the insulin. Tests were done at weekly intervals. Four animals were used and each animal was tested 3 times with both insulins. The difficulty of making an accurate assay of insulin is well known and a definite strength cannot be ascribed to this sample of human insulin on the basis of the small number of tests done. However, all the tests indicated that the sample contained less than 30 U per ml. and it may have contained as little as 20 U per ml. On the basis of the tests done, a potency of 25 U per ml. was assigned to it but this must be regarded as only approximate. The intravenous injection of 0.6 ml. of this preparation in a fasting non-diabetic individual was followed by a fall in blood sugar from 106 mgm. per 100 ml. to 78 mgm. per 100 ml. in 45 minutes, with a subsequent rise to the fasting level, 90 minutes after the test was started. This fall in blood sugar is not large and suggests that the preparation contained less than 25 units per ml.

A severe generalized allergic reaction followed the intravenous injection of human insulin in this patient on a previous occasion (2). Therefore, later in the day (November 13) on which the insulin tolerance test with 30 units of crystalline insulin was done, the patient was given graded doses of human insulin for desensitization. Injections were first made endermally, then subcutaneously, and finally intravenously. A total of approximately 50 units (2 ml.) of the preparation of human insulin was given in a period of about 6 hours. One hour after the last dose, the patient became weak and shaky. Orange juice gave relief. On the following day (November 14), the fasting blood sugar was 282 mgm. per 100 ml. The injection of 25 units (1.0 ml.) of human insulin intravenously was followed by a fall in the blood sugar to 122 mgm. per 100 ml. in 2 hours. There was no allergic reaction during this test. The results of the tests with crystalline and human insulins are shown in Figure 2.

Finally, on November 15, 1942, 60 units of regular pork insulin (Lilly) were given intravenously over a period of 35 minutes. The blood sugar was 310 mgm. per 100 ml. before the injections and at the end of 90 minutes was 278 mgm. per 100 ml. During this hospital admission, the patient received approximately 1500 units of insulin. She was discharged on the 17th hospital day with instructions to follow a diet and to take no insulin. Diagnoses: Diabetes mellitus; diabetic retinitis; resistance to insulin;

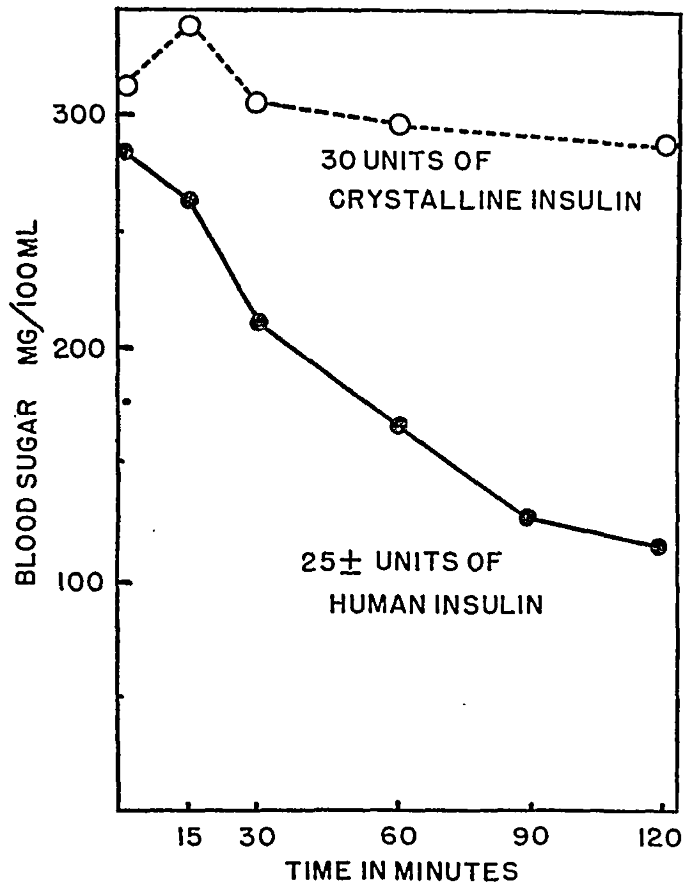


FIG. 2. INTRAVENOUS INSULIN TOLERANCE TESTS

allergy to insulin; peripheral neuritis; chronic pyelonephritis, *E. coli;* obesity.

DISCUSSION

This patient differs from the reported cases of insulin resistance in that she received relatively small amounts of insulin and there were long periods during which no treatment was given. Continuous administration of the huge doses of insulin usually required for resistant patients was not attempted because of the marked local and systemic allergic reactions and because of the poor prospect of benefit to the patient. Withholding of insulin was possible because a fair state of health could be maintained on diet alone.

There were 3 periods during which the patient received insulin and exhibited resistance. On 2 of these occasions, resistance was preceded by a very brief state of relative responsiveness to insulin which, in the first instance (February 20, 1942), was demonstrated on the third day, and in the second (November 3, 1942), on the fifth day after desensitization with insulin was begun. The greater length of time required in the second instance was due to the mistake in believing that 10 units given daily would effect desensitization. No test for determining the responsiveness of the patient could be done until preliminary desensitization had been carried out. In each case, the temporary state of susceptibility to insulin was preceded by a period of 5 months or more during which the patient received no insulin whatsoever. *It appears probable, therefore,* that during the extended periods without treatment the resistance became less or disappeared. Resistance reappeared within about 10 to 12 days of beginning densensitization and persisted as long as insulin was given. Temporary responsiveness was not observed during the admission to the Boston City Hospital in the fall of 1941. This may have been due to inadequate dosage of insulin within the first 7 to 10 days of beginning desensitization.

The patient's course during the period of study is illustrated in Figure 3. This shows the relationship between the administration and withholding of insulin and the changes observed in the patient's resistance. The results of tests for insulin-neutralizing activity (I.N.A.) in the patient's serum are also indicated. These tests were carried out in mice and are described in detail in the second part of this study (5). A plus sign indicates the presence and a zero the absence of insulin-neutralizing activity in the serum. When the patient was demonstrably resistant to insulin, the blood showed insulin-neutralizing activity but blood obtained when the patient was responsive to insulin, or shortly beforehand, showed none. Neutralizing activity was apparently present in bloods obtained as long as 10 weeks after the administration of insulin had been stopped.

YEAR	1941					1942											
MONTH	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11
RESISTANCE	+						0	+								0 +
I.N.A.	+		+			0	0	+	+	+			0		0	0 +
INSULIN	GIVEN	WITHHELD					GIVEN		WITHHELD							GIVEN

FIG. 3. CHANGES IN THE DEGREE OF INSULIN RESISTANCE AND OF INSULIN NEUTRALIZING ACTIVITY (I.N.A.) OF THE SERUM IN RELATION TO THE ADMINISTRATION OF INSULIN

These observations are consistent with the view that resistance to insulin in this patient was immunologic in nature and that the insulin-neutralizing activity of the patient's serum was due to the presence of a neutralizing antibody for insulin. This is also supported by the patient's greater responsiveness to human than to crystalline insulin, indicating some degree of specificity in the resistance. Tests in mice also indicated a similar specificity (2, 5). Immunologic identity for insulins derived from a number of mammalian species including man has been claimed (6). This conclusion was reached on the basis of cross reactions in complement fixation tests and anaphylaxis in guinea pigs. However, it is commonly agreed that cross reactions in immunologic systems, done without quantitative control, indicate similarity but do not prove identity.

Resistance to insulin may not always be demonstrably specific. A second patient, C. S.,[1] was found to be resistant to both human and commercial insulins. This was a 60-year-old white female who also had lipodystrophy. She had required daily doses of insulin ranging from 500 to 2,500 units for a period of 9 months. Injection of the large doses of insulin required for control of the diabetes usually caused some local redness and when extremely large doses were given, a few hives occasionally appeared. A single dose of approximately 50 units (2 ml.) of the same preparation of human insulin given the first patient, A. M., was injected intravenously in the fasting state. This caused no discomfort but 3 hives appeared on the thighs and arm. The blood sugar was 274 mgm. per 100 ml. before the injection and fell gradually to 247 mgm. per 100 ml. at the end of 2 hours. This fall was not considered significant. The blood of this patient also showed insulin-neutralizing activity when tested with crystalline (beef and pork) insulin, and this was also demonstrable in tests with human insulin.

One explanation for the difference in the responses of the 2 insulin resistent patients to human insulin was the development of a less specific antibody in the second patient than in the first. An analogy is seen in experiments by Hooker and Boyd (7, 8) which were carried out in rabbits. For example, early in the course of injections of chicken ovalbumin, antibody of a high degree of specificity was produced. When the injections of antigen were continued and the animals became "hyper-immune," the antibody then precipitated duck ovalbumin, a related antigen. To return to the 2 insulin resistant patients, the first, A. M., received approximately 5,000 units of insulin over a period of about 14 months, whereas the second received an estimated total of about 200,000 units in 9 months, 40 times the first figure. Thus, compared to the first patient, the second might be considered to be "hyper-immune" with respect to insulin.

Marked allergy to insulin as manifested by a tendency to generalized urticaria and constriction in the chest was not observed to be associated with resistance, and the conclusion seems warranted that the two were distinct and possibly independent immunologic mechanisms. For example, on 2 occasions in the clinical course of A. M. (February and October 1942), a high degree of allergy was observed only a few days before responsiveness to insulin was demonstrated. This suggests that the allergic state occurred in the absence of resistance. The objection may be raised that a test for susceptibility to insulin was never carried out *until* desensitization was accomplished and it may be argued that desensitization itself was the cause of the reduced resistance. The rôle of desensitization in inducing responsiveness may be doubted, however, because resistance was observed while the patient was still in the desensitized state. If resistance and the state of desensitization could coexist, it seems unlikely that the latter would also bring about the disappearance of the former. The failure to demonstrate the insulin-neutralizing factor in the blood before desensitization was begun, is added evidence for the view that allergy could exist in this patient in the absence of resistance. If this is correct, the development of resistance in A. M. must be attributed to something other than allergy to insulin.

A theory which explains many of the features exhibited by A. M. is given below. Two distinct antibodies for insulin are postulated (2, 5, 10).

[1] Opportunity to study this patient in the Deaconess Hospital, Boston, was kindly afforded by Dr. Howard F. Root.

One of these is the insulin-neutralizing factor which is considered to be analogous to, but not necessarily identical with, antibody produced in animals following the injection of a protein. Production of antibody of this type may be induced by injections of antigen and when the injections are stopped, the titer falls slowly. The second antibody is the allergic antibody, also referred to as atopic reagin and is only demonstrable in the skin of suitable recipients (Prausnitz-Küstner reaction). An important peculiarity of this antibody is that once it makes its appearance in the blood, it may persist in undiminished titer for long periods without antigenic stimulus (9).

The proposed theory is represented schematically in Figure 4. A complete cycle is shown

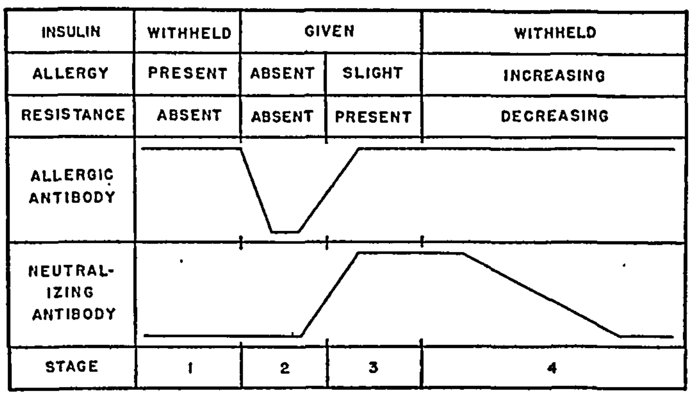


Fig. 4. Schematic Representation of the Suggested Relationship Between the Allergic and the Resistant States in A. M.

illustrating 4 consecutive stages: (*1*) a high degree of allergy; (*2*) desensitization with responsiveness; (*3*) desensitization with resistance; (*4*) the return of the allergic state and the loss of resistance. The period from January to October 1942 (Figure 3) represents such a cycle. Before desensitization, the allergic antibody is present and the neutralizing factor is absent (stage I, Figure 4). Allergic manifestations are easily induced and it is assumed that resistance is not present at this time. With increasing doses of insulin, neutralization of the allergic antibody takes place in the patient. The titer falls and desensitization is accomplished. The neutralizing antibody has not made its appearance and relatively small doses of insulin can induce hypoglycemic reactions. This may be called the first phase of desensitization. The second phase (stage *3*) is characterized by the appearance of the insulin-neutralizing factor as a result of the antigenic stimulus. There are 2 consequences: (*1*) reappeance of resistance; (*2*) a return of the allergic antibody, but without the development of systemic manifestations of allergy. This is explained by the greater avidity (10) of the neutralizing antibody for the antigen as compared with the allergic antibody, with the result that the former combines preferentially with insulin. It is assumed that this affords protection by preventing un-neutralized insulin from reaching the sensitized cells of the body. Systemic manifestations of allergy can now be produced only if large amounts of insulin are given (see above, hospital admission of August 1941). When the administration of insulin is stopped and no further antigenic stimulus occurs (stage 4), the neutralizing factor slowly disappears. The allergic antibody persists, however, and the patient finally returns to the allergic state and may again exhibit responsiveness to insulin after desensitization. The cycle is now complete.

This theory can also explain the marked systemic allergic reaction which occurred in A. M. in the first tolerance test with human insulin (2). This was done at a time when she was desensitized and resistant to *crystalline* insulin. After injection of the human preparation, generalized urticaria occurred which was followed by a hypoglycemic reaction, another example of the presence of allergy in the absence of resistance to insulin. Neutralizing activity for crystalline but not for human insulin was demonstrable in serum taken at that time (2). However, sensitization of normal skin to both human and crystalline insulin was easily accomplished. The allergic reaction as well as the lack of resistance may be ascribed to the absence of a neutralizing antibody for human insulin with the result that neutralization did not occur, and insulin remained free to produce allergic symptoms. If this explanation is correct, one may also infer that the allergic antibody was less specific than the neutralizing factor.

SUMMARY AND CONCLUSIONS

A case of resistance to insulin, associated with a high degree of allergy to insulin, is reported.

Changes occurred in the degree of resistance which were apparently related to the giving and withholding of insulin. The resistance was specific in that human insulin caused a markedly greater fall in the blood sugar than did crystalline insulin. The findings in this patient support the view that the resistance to insulin is immunologic in nature.

The clinical course and the experimental findings indicate that allergy to insulin and resistance to insulin varied independently of each other. Two phases of desensitization were found, the first characterized by the absence, the second by the presence of resistance to insulin. It is suggested that the clinical observations may be explained by postulating 2 immune systems: (*1*) the allergic mechanism associated with the skin-sensitizing antibody, responsible for generalized urticaria, constriction in the chest, and collapse; (*2*) the insulin neutralizing mechanism associated with a neutralizing antibody for insulin and responsible for the patient's resistance to insulin.

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IMMUNOLOGIC STUDIES IN INSULIN RESISTANCE

II. THE PRESENCE OF A NEUTRALIZING FACTOR IN THE BLOOD EXHIBITING SOME CHARACTERISTICS OF AN ANTIBODY

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The huge doses of insulin required by some insulin-resistant patients have led many observers to postulate the presence of an insulin-neutralizing factor in the blood of such individuals. In attempts to demonstrate this factor, the usual procedure has been to inject insulin mixed with the patient's serum into laboratory animals and then to look for a decrease or absence of insulin effect. Some studies have given entirely negative results (1 to 4), possibly because of the large amounts of insulin used in carrying out the tests. However, a few workers have obtained evidence indicating some insulin-neutralizing activity (5 to 7).

The interpretation of experiments to be reported here, as well as those reported by others, is made difficult by the wide variations in response to insulin exhibited by different animals and even by the same animal at different times. Also, factors other than the injection of insulin may possibly affect the animal's response. Irritation from the injected foreign protein (human serum), the presence of high concentrations of glucose in the injected serums, and delay in the absorption of the insulin (if mixture is not injected intravenously), may all combine to interfere with insulin effect and produce the impression that neutralization has taken place.

Earlier experiments in mice with blood obtained from a case of insulin resistance, A. M., provided evidence for the presence of an insulin-neutralizing factor (8, 9). It was believed at that time that this was an antibody. Additional evidence has been obtained from a study of a series of 25 blood specimens obtained from the same patient. Tests have also been made with blood from a second insulin-resistant patient. These studies leave little doubt that an insulin-neutralizing factor was present and that this factor was an antibody for crystalline insulin.

TESTS FOR INSULIN-NEUTRALIZING ACTIVITY

Materials and methods

Adult albino mice were starved for 24 hours without bedding but with access to water. Each animal was then injected intra-abdominally with 0.5 ml. of a mixture of 2 or more of the following: U 40 crystalline insulin (Lilly), saline, human serum, and glucose. The mice were observed continuously for 90 minutes in an incubator measuring approximately 7 × 4 × 5 feet. The thermostats were set for a temperature of 35° C. to 37.5° C., but because the incubator door was opened at intervals during the tests, the temperature averaged slightly lower than this. The number of mice showing insulin effect was recorded. No animal was used more than once.

Insulin effect was considered to be present when one or more of the following symptoms appeared: convulsions, extreme irritability and overactivity, coma, loss of equilibrium, and marked weakness or paralysis of the hind legs. These symptoms disappeared quickly when a glucose solution was injected intra-abdominally. During the test, the mice were kept under continuous observation because sumptoms of insulin effect were sometimes transitory.

All dilutions of insulin were freshly made. The human serum was obtained from clotted blood and stored under sterile conditions in rubber-stoppered test tubes in the ice-box. The mixtures of insulin and serum were not incubated before the tests were done. In most instances, 5 mice were injected with each mixture. This small number was used because the amount of serum was limited. Glucose determinations on the serums from normal and insulin-resistant patients were made by the Folin micromethod, using serum instead of whole blood. Most of these determinations were made within a short period after the serums were tested in mice.

Experiments with normal human serum

The tests with normal serum were originally designed as controls for some of the experiments with serums from the 2 insulin-resistant patients. However, for purposes of presentation, tests of

a similar nature are grouped together. The dates on which the different tests were done are given in the tables. Thus tests done on the same day may be found in the tables and compared if desired.

A total of 10 tests, in which 5 mice were used in each, were carried out with 5 normal serums. The results are shown in Table I. Solutions for injection were made by mixing 2.5 ml. of human serum, with 0.5 ml. of U 40 crystalline insulin diluted 1 : 100. Each mouse was injected with 0.5 ml. which contained 0.033 units of insulin, approximately 0.4 ml. of serum and 0.1 ml. of saline. The incidence of symptoms varied, but in all tests, 2 or more of the 5 mice in each group showed evidence of insulin effect. In this group of 50 mice, 40 showed symptoms, an incidence of 80 per cent, which is below that observed in experiments done without the addition of serum. The greater resistance of the mice to insulin in the experiments shown in Table I, compared to that noted in the first report (8), may have been due to the lower temperature at which the more recent tests were carried out.

Tests in 54 mice were also done with normal serum to which sufficient glucose was added to raise the concentration to the level expected in serums obtained from insulin-resistant diabetic patients. The final concentration attained after the addition of glucose was equivalent to a blood sugar of between 240 and 300 mgm. per 100 ml. The solution containing insulin and serum was made in the proportions described above but

TABLE I

Tests with normal serum

Number of test	Serum	Date		Number of mice showing	
		Serum taken	Of test	Symptoms	No symptoms
1	A	November 1, 1941	April 8, 1942	5	0
2	B	November 7, 1941	October 27, 1942	4	1
3	A	November 8, 1941	August 13, 1942	4	1
4	A	November 12, 1941	November 6, 1942	2	3
5	C	December 6, 1941	August 26, 1942	4	1
6	C	December 6, 1941	August 27, 1942	5	0
7	D	February 2, 1943	April 20, 1943	5	0
8	D	February 2, 1943	April 27, 1943	3	2
9	D	February 2, 1943	April 29, 1943	3	2
10	E	March 24, 1942	August 11, 1942	5	0
Total (50 mice)				40	10
Incidence of symptoms = 80 per cent					

TABLE II

Tests with normal serum with added glucose

Number of test	Serum	Date		Glucose content	Number of mice showing	
		Serum taken	Of test		Symptoms	No symptoms
				mgm. per 100 ml.		
1	D	February 2, 1943	April 27, 1943	240	1	4
2	D	February 2, 1943	April 29, 1943	240	2	3
3	D	February 2, 1943	May 8, 1943	240	7	0
4	D	February 2, 1943	May 11, 1943	240	3	2
5	D	February 2, 1943	May 13, 1943	240	3	2
6	D	February 2, 1943	June 4, 1943	240	2	3
7	G	June 28, 1943	July 8, 1943	300	13	9
Total (54 mice)					31	23
Incidence of symptoms = 57 per cent						

with the addition of 0.3 ml. of a 2 per cent glucose solution for each 3.0 ml. of the mixture of serum, saline, and insulin. Five-tenths ml. was injected into each of 5 or more mice. Each mouse received, therefore, 0.0303 units of insulin, approximately 0.4 ml. of serum, 0.1 ml. of saline, and 0.91 mgm. of added glucose. In 7 tests done with normal serum to which glucose was added, the incidence of symptoms was again very variable (Table II). In 1 test, only 1 mouse showed symptoms. Of 54 mice tested in this way, 31 showed symptoms, an incidence of about 57 per cent.

These results indicate that under the experimental conditions and with high concentrations of glucose in the serum, an incidence of symptoms of somewhat over 50 per cent may be expected. Because of the increased volume of the injection mixture, due to the addition of glucose, the dose of insulin in these tests was 0.003 units less than the tests shown in Table I and those to be described below done with serums from the 2 insulin-resistant patients. It is probable that this slight reduction in dose was not responsible for the decreased incidence of symptoms in tests done with added glucose.

Experiments with serum from 2 insulin-resistant patients

The clinical course of the first insulin-resistant patient, from whom serums were obtained for these studies, is reported in detail elsewhere (10). This patient, A. M., was observed for about 15

months and bloods taken at intervals during this period were tested for insulin neutralizing activity. Mice were injected with mixtures of 2.5 ml. of the patient's serum, diluted or undiluted, and 0.5 ml. of U 40 insulin diluted 1 : 100, as in the tests with normal serum. No glucose was added. Each mouse received therefore 0.033 units of insulin, 0.40 ml. or less of serum, and 0.1 ml. or more of saline.

The results of 24 tests on 21 serums are shown in Table III. The serums are listed in the order in which they were taken and the dates are given, as well as the dates on which the tests were done. The concentrations of glucose in all but 4 of the serums are also shown. Five mice were used in each test, and 3 serums were tested twice. The regular alternation in the results of the tests is striking. In the first 4 tests, no mice showed

TABLE III

Results of tests with 22 serums obtained from A. M. over a period of 14 months

Number of test	Date: Serum taken	Date: Of test	Concentration of glucose (*mgm. per 100 ml.*)	Number of mice showing* symptoms	Resistance	Remarks
1	August 19, 1941	August 13, 1942	279	0		
2	August 21, 1941	August 21, 1942		0	+	Patient receiving insulin
3	August 28, 1941	August 13, 1943	340	0		
4	October 8, 1941	August 13, 1943		0		
5	January 21, 1942	August 13, 1943	332	1	?	No insulin given from September 7, 1941 to February 17, 1942
6	February 11, 1942	August 11, 1942	365	3		
7	February 11, 1942	August 21, 1942	365	4		
8	February 21, 1942	August 21, 1942	227	3	0	
9	February 27, 1942	August 11, 1942	354	0		Insulin given from February 18, 1942 to March 12, 1942
10	March 2, 1942	August 11, 1942	389	0	+	
11	March 11, 1942	August 21, 1942	393	0		
12	April 6, 1942	August 13, 1942	286	0		
13	May 27, 1942	August 11, 1942	469	0		No insulin given from March 13, 1942 to October 29, 1942
14	August 18, 1942	May 11, 1943		1		
15	August 18, 1942	April 27, 1943	244	2	?	
16	October 30, 1942	November 6, 1942	280	2		
17	November 2, 1942	November 6, 1942	319	3		
18	November 4, 1942	November 6, 1942	42	3	0	
19	November 6, 1942	June 4, 1943	275	1		
20	November 8, 1942	June 4, 1943	298	0	?	Insulin given from October 30, 1942 to November 15, 1942
21	November 9, 1942	May 13, 1943	305	0		
22	November 9, 1942	June 4, 1943		0		
23**	November 10, 1942	May 13, 1943	202	0	+	
24**	November 12, 1942	May 13, 1943	297	0		

* 5 mice were used in each test. ** Serum diluted 1 : 2.

symptoms and this is also true of the ninth to the thirteenth and the twentieth to the twenty-fourth tests, inclusive. On the other hand, one or more mice showed symptoms in the fifth to the eighth and the fourteenth to the nineteenth tests. In 8 of the 10 tests in which one or more mice showed symptoms, the serum contained 227 mgm. per 100 ml. of glucose or more. This indicates that the presence of high concentrations of glucose in the serums was not the determining factor in the prevention of symptoms in tests in which no symptoms occurred. The results of these tests will be discussed below in relation to the patient's clinical course.

Seven tests done with a total of 40 mice with a serum obtained on November 19, 1942, 5 days after all injections of insulin had been stopped, are shown in Table IV. This serum was

TABLE IV

Tests with serum obtained from A. M. on November 19, 1942

Number of test	Date of test	Dilution of serum	Number of mice showing		Incidence of symptoms
			Symptoms	No symptoms	
					per cent
1	April 20, 1943	1 : 2	0	5	
2	May 4, 1943		0	5	
3	May 8, 1943		0	10	
4	June 19, 1943		0	5	
	Total (25 mice)		0	25	0
5	April 20, 1943	1 : 4	0	5	
6	April 29, 1943		1	4	
7	August 4, 1943		1	10	
	Total (21 mice)		2	19	9.5
8	August 5, 1943	1 : 8	4	7	37

diluted 1 : 2 in 4 tests, 1 : 4 in 3 tests, and 1 : 8 in 1 test. Of 30 mice tested with serum diluted 1 : 2, none showed symptoms. Of 21 mice tested with serum diluted 1 : 4, 2 showed symptoms. Four of 11 mice receiving serum diluted 1 : 8 showed symptoms. In comparing the results of tests with undiluted normal serum and those obtained with diluted serums, account should be taken of the tendency of undiluted normal serum to decrease the incidence of symptoms. This non-specific inhibition of insulin is

TABLE V

Results of tests with serum obtained from C. S.

Number of test	Date test done	Dilution of serum	Number of mice showing		Incidence of symptoms
			Symptoms	No symptoms	
1	August 10, 1943	Undiluted	2	9	*per cent*
2	August 26, 1942	1 : 2	0	3	
3	August 27, 1942		0	5	
4	August 6, 1943		3	8	
	Total (30 mice)		5	25	17
5	August 26, 1942	1 : 4	2	3	
6	August 27, 1942		3	2	
	Total (10 mice)		5	5	50

decreased or absent in tests with serums diluted 1 : 2 or more. Furthermore, the "glucose effect" in such tests is also diminished.

A few tests were done on a serum (Table V) obtained from a second insulin-resistant patient whose clinical course has also been briefly described (10). Two of 11 mice tested with undiluted serum showed symptoms. Of 19 mice tested with serum diluted 1 : 2, 3 developed symptoms. Five mice out of a total of 10 showed symptoms when the serum was diluted 1 : 4. These results indicate that the insulin-neutralizing capacity of serum from this patient was less marked than that of the serum obtained from A.M. on November 19, 1942 (Table IV).

Serums from these 2 patients were also tested for their ability to protect mice from the same preparation of human insulin which was used in the insulin tolerance tests. This preparation has been described in the first part of this study. One patient, A. M., exhibited susceptibility to this preparation at a time when she was resistant to crystalline insulin. The other, C. S., was apparently resistant to human as well as crystalline insulin (10). The potency of this preparation of human insulin was approximately 25 units per ml. In the tests with mice, 0.5 ml. of this diluted 1 : 75, was added to 2.5 ml. of serum diluted 1 : 2. The estimated dose per mouse was slightly less than 0.03 units. The

TABLE VI

Serum from A. M. and C. S. tested with human insulin

Patient	Date serum taken	Date of test	Dilution of serum	Number of mice showing		Incidence of symptoms
				Symptoms	No symptoms	
						per cent
A. M.	November 19, 1942	May 5, 1943 June 19, 1943 June 29, 1943	1 : 2	1 3 8	4 2 3	
	Total (21 mice)			12	9	57
C. S.	August 23, 1942	August 5, 1943 August 6, 1943	1 : 2	1 2	10 9	
	Total (21 mice)			3	19	14

results of the tests are shown in Table VI and may be compared with similar tests with crystalline insulin in Tables IV and V. The results indicate that the serum obtained from A. M. on November 19, 1942, was less active in preventing symptoms in mice injected with human than with crystalline insulin. A dilution of 1 : 2 failed to protect against human insulin whereas a dilution of 1 : 2 gave solid protection, and 1 : 4 gave some protection against crystalline insulin. This result is consistent with the patient's greater susceptibility to human insulin (10). The serum of C. S. gave about equal protection against both insulins. This patient was resistant to human as well as crystalline insulin (10).

TESTS FOR SKIN SENSITIZING ANTIBODY

Earlier studies (8) indicated that the antibody responsible for passive sensitization of normal human skin was distinct from the insulin-neutralizing factor. A few further studies have been made on this point.

Four serums obtained from A. M. were diluted serially in steps of 2 and the dilutions were injected endermally in a recipient known to receive passive transfer well. The sites were tested 24 hours later with 0.02 ml. of a 1 : 10 dilution of U 40 crystalline insulin (Lilly). Serums obtained on August 19, 1941, January 21, 1942, and February 11, 1942, gave titers of 1 : 32 and the serum of October 9, 1941, a titer of 1 : 16. Therefore, little or no change in the amount of skin-sensitizing antibody in the patient's serum occurred over a period of about 6 months. Tests for insulin-neutralizing activity (Table III, tests numbers 1, 4, 5, 6, and 7) show that a decrease in this occurred during that period. Blood taken on February 21, 1942, 3 days after desensitization with insulin was begun and when responsiveness to insulin was present, caused weak sensitization of normal skin in a dilution of 1 : 4, and none in a dilution of 1 : 8. This decrease may have been caused by the desensitization. Attempts to demonstrate skin-sensitizing antibody in the serum of C. S. were unsuccessful, although insulin-neutralizing activity was present.

OTHER TESTS FOR ANTIBODY

In Table VII are shown the results of tests for precipitins by the collodion-particle method (11).

TABLE VII

Tests for precipitins in serums obtained from A. M.

Date serum obtained	Titer of precipitins	I.N.A.*	Resistance to insulin
August 25, 1941	1 : 32		+
August 27, 1941	0		+
August 28, 1941	1 : 4	+	+
September 2, 1941 A.M.	0		+
September 2, 1941 P.M.	1 : 16		+
February 21, 1942	0		0
February 27, 1942	0	+	?+
March 11, 1942	0	+	+

* Insulin neutralizing activity. See Table III, tests number 3, 9, and 11.

Crystalline insulin (Lilly) was used as the antigen. Of 8 serums tested, 3 showed precipitins. All the serums, barring that of February 21, 1942, and possibly that of February 27, 1942, were obtained from A. M. at a time when she was resistant to insulin. On the basis of other studies made on this patient (Table III), it may be assumed that 6 of these serums contained the insulin-neutralizing factor, although only 3 were tested for its presence (Table III, tests number 3, 9 and 11).

Two serums (August 25, 1941 and March 11, 1942) were tested in normal skin and conferred sensitivity to insulin on the injected sites. It is probable that most if not all of the other 6

serums listed in Table VI also contained the skin-sensitizing antibody (see above).

The serum obtained from A. M. on November 19, 1942, was tested for the presence of complement-fixing antibodies and for precipitins by the ring test. The pH of the insulin was adjusted to about 7.0 in order to avoid non-specific precipitation. No antibody was demonstrated by either method. This serum was chosen because it had been thoroughly tested in mice (Table IV) and insulin-neutralizing activity was clearly demonstrated in a titer of 1 : 2 and was probably present in a dilution of 1 : 4. No precipitins were demonstrable by the ring test in the serum obtained from C. S.

DISCUSSION

Because of the small number of animals used and the relatively low incidence of symptoms (57 per cent) in the control tests, the interpretation of a single test for insulin-neutralizing activity in serum is subject to error. On the basis of chance, it might occasionally happen that a group of 5 mice would be selected in which no animal would show symptoms, even though neutralization of insulin had not taken place. Therefore, absence of symptoms in a group of 5 mice is indicative of insulin-neutralization but cannot be taken as conclusive evidence thereof. On the other hand, the appearance of symptoms in one or more mice in a group of 5 is a reliable indication that neutralization of insulin was incomplete or absent.

Consideration of the results of the tests with serums obtained from A. M. in relation to the patient's clinical course (10) is of interest. As shown in Table III, no symptoms appeared in any of the mice tested with serums obtained on the 3 occasions when the patient was demonstrably resistant to insulin (tests number 1, 2, 3, 9, 10, 11, 21, 22, 23, and 24). The same result was obtained with serums drawn within a period of 8 weeks after resistance was demonstrated and the administration of insulin had been stopped (tests number 4, 12, and 13). In this entire group, no symptoms developed in a total of 65 mice tested. A different result was obtained with serums taken when the patient was responsive to insulin (tests number 8, 18, and 19), as well as serums taken within a period of approximately 3 months or less before responsiveness to insulin was demonstrated (tests number 5, 6, 7, 14, 15, 16, and 17). In this group of 50 mice, 23 showed symptoms, an incidence of 46 per cent. The appearance and disappearance of insulin-neutralizing activity in the patient's serum was apparently dependent on whether or not the patient received insulin. During 2 long periods of about 5 and 7 months (September, 1941 to February, 1942, and March, 1942 to November, 1942), when the patient was receiving no insulin, the neutralizing activity of the serum disappeared. At the end of these periods (February, 1942, and November, 1942), when resistance to insulin was markedly diminished or absent, the administration of insulin was followed in about 10 to 12 days by the reappearance of neutralizing activity in the serum and the development of resistance to insulin. These results are in accord with the view that the factor in the patient's serum responsible for the prevention of symptoms in mice was a neutralizing antibody for crystalline insulin.

The method used for the demonstration of neutralization of insulin did not lend itself to quantitative measurements. Rough estimates of the amount of the insulin-neutralizing factor, made by diluting the serum, showed that only small amounts were present. A dilution of 1 : 4 resulted in some loss of protection in tests with serum from A. M. (Table IV) and complete loss in tests with serum from C. S. (Table V). The smaller capacity of the second serum to neutralize insulin may have been due to a reduction in the circulating antibody to insulin, caused by the large doses of insulin which the patient was receiving daily.

The patients are compared in Table VIII with respect to the results of the tests for insulin-neutralization and with respect to their resistance to the 2 insulins, as indicated by tolerance tests (10). It is interesting that the serum of A. M. exhibited species specificity. In a dilution of 1 : 2, it failed to neutralize human insulin (Table VI) although neutralization of crystalline insulin was marked. Specificity was not demonstrated in tests with serum from C. S. The difference in the behavior of these serums may be

TABLE VIII

Comparison of A. M. and C. S. with respect to clinical features and results of tests in mice with serum diluted 1 : 2

Serum	Insulin resistance		Incidence of symptoms in mice		Remarks
	Crystalline	Human	Crystalline	Human	
			per cent		
A. M. November 19, 1942	+++	0	0	57	Serum taken after 5 days without insulin
C. S. August 23, 1942	+++	+++	17*	14	Patient receiving approximately 1000 units daily with partial control of the diabetes

* One test done with undiluted serum.

explained by assuming that an antibody of wider reactivity was developed by C. S. than by A. M. This is discussed in the first part of this study in relation to the clinical histories (10).

The relationship of precipitins and complement fixing antibodies to insulin-resistance is not clear at the present time. No correlation was observed between the presence of precipitins in the serum of A. M. and either insulin-neutralizing activity or skin-sensitizing antibody (Table VII). Goldner and Ricketts (12) described 4 patients who were allergic to insulin and whose serum contained precipitins demonstrable with the collodion particle method. Two of these patients were also resistant to insulin. One of the 2 was extremely resistant and this patient's serum contained precipitin in a titer of 1 : 80. The serum of 3 other allergic patients, one of whom was insensitive to insulin, contained no precipitins. In animal experiments (13), the development of complement-fixing antibody was not associated with the appearance of resistance to insulin.

In a previous report (8), the conclusion was reached that the factor responsible for neutralization of insulin, and the antibody demonstrable by passive transfer to normal skin (Prausnitz-Küstner reaction), were not the same. This is supported by the tests made with 4 serums from A. M., which showed that the insulin-neutralizing factor and the skin-sensitizing antibody did not vary in a parallel fashion. Furthermore, the serum of C. S. protected mice from insulin but did not sensitize human skin to insulin.

SUMMARY

A method for demonstrating neutralization of insulin by serum obtained from insulin-resistant subjects is described. The results of tests on a number of serums obtained from 2 insulin-resistant patients showed that the presence of insulin-neutralizing activity was associated with resistance to insulin. The factor responsible for neutralization of insulin in the serum of one patient exhibited species specificity, and appeared to vary independently of the skin sensitizing (allergic) antibody.

It is concluded that: (*1*) insulin resistance may occur on an immunologic basis and may be associated with the presence in the serum of a neutralizing antibody for crystalline insulin; (*2*) under certain circumstances, this antibody may exhibit species specificity; (*3*) the insulin-neutralizing and the skin-sensitizing antibodies are distinct.

This study was carried out with the technical assistance of Miss Lucie Pray.

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A STUDY OF CAPILLARY PERMEABILITY IN EXPERIMENTAL BURNS AND BURN SHOCK USING RADIOACTIVE DYES IN BLOOD AND LYMPH[1]

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The integrity of the capillary membrane is essential to the maintenance of the circulation in a normal animal. Increased loss of plasma colloids through the capillary wall may be an important factor in the development of a pathologic state. In recent years, interest has centered upon abnormal capillary permeability, and local changes in permeability have been demonstrated in areas of trauma and burn. A generalized increase in capillary permeability has been postulated in shock, but the evidence is conflicting. Known to occur when histamine is injected intravenously (1), it might take place in other types of shock and has been suggested as a reason for the irreversibility of late shock. The tissue anoxia that occurs as a result of prolonged low blood pressure and decreased blood flow in shock could result in an increase in capillary permeability which would not be limited to the site of injury.

Direct quantitative measurement of capillary permeability presents technical difficulties because of the microscopic character of the spaces involved. Several workers, including Krogh (2) and Landis (3), have succeeded in experimental animals in observing directly through a dissecting microscope the passage of dyes, particulate matter, and blood cells out of the capillary into the extravascular space. Landis (3), using a micro-pipette, has been able to measure capillary pressure in both animals and man. Such observations, while demonstrating that under experimental conditions the capillary wall is permeable to large molecules and even particulate matter, have not shown to what extent, in the intact animal with capillaries undisturbed by manipulation, the capillary is permeable to colloids and at what rate this exchange of colloids across the capillary wall takes place.

Much of our knowledge of capillary permeability is the result of the use of indirect methods. The local concentration of colloidal dyes (4) or radioactive proteins (5) in tissues after injection of these substances into the blood stream has furnished information. Permeability has also been estimated by the flow and protein concentration of lymph. In 1932, Field, Drinker, and White (6) found that the flow of lymph from dogs' feet was augmented after immersion in hot water, revealing an increased filtration of plasma fluid and protein from the capillary into the interstitial space and thence into the lymphatic vessel. This local change in permeability in burns has been amply confirmed recently by Glenn, Petersen, and Drinker (7), and Glenn, Muus, and Drinker (8).

Neither of the two types of indirect method quoted has proven altogether satisfactory in the determination of capillary permeability. The use of the concentration of colloids in excised tissue may be misleading because of the difficulty of removing the blood from the tissue without also removing the extravascular, interstitial fluid. The method of Drinker, employing lymph flow, protein concentration, and their product as a measure of permeability, is open to doubt under conditions of reduced blood flow. The concentration of protein in the interstitial fluid (and therefore in the lymph) theoretically can be increased by absorption of fluid at the venous end of the capillary without any change in capillary permeability. And a decreased amount of protein may be found in the lymphatic trunk in the presence of increased capillary permeability if the blood flow is sufficiently reduced. Blood flow, except in an area of trauma, is commonly reduced in shock.

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[2] Fellow in the Medical Sciences, National Research Council.

Recently, Tobin and Moore (9) have synthesized radioactive colloidal dyes which by means of their radioactivity permit a more sensitive measurement of dye concentration.[3] It was decided to adapt this dye technic to the study of capillary permeability by the simultaneous measurement of the radioactive concentrations in blood stream and lymph. Certain di-azo acid dyes form a chemical union with protein (11) and, when injected into the blood stream, rapidly combine with the plasma protein (12). Radioactive derivatives of these dyes also form this protein bond (9), and, in the blood stream, represent tagged molecules; the rate of their appearance in the lymph should prove an effective measure of capillary permeability.

Since the sole source of radioactivity in such an experiment is the blood stream into which the radioactive dye has been injected, the rate of appearance of radioactivity in the lymph must be a function of capillary filtration and cannot be the result of the accumulation of stagnant protein in the interstitial space.

In this paper are reported both the details of the method and the results obtained in experimental burns.

METHODS

A "tagged" colloidal dye, which becomes associated with protein in the serum, is used. This substance is injected into the blood stream and its slow permeation across the capillary into lymph measured, in samples of lymph, by a physical detection apparatus. Simultaneously, its disappearance from the serum is measured. Thus, a set of curves is obtained, one for serum decreasing with time, and several for lymph, from various sources, increasing with time. The relationship of these curves to each other is a direct quantitative measurement of the permeability of the capillaries from which the lymph flows.

The radioactive colloidal dyes used have been brominated derivatives of trypan blue and Evans blue (T-1824). Most of the experiments were done with the latter. The radioactive di-brom dyes have previously been described and their synthesis and properties, as well as measurement technics, have been discussed (9, 10, 13). The radioactivity is due to the presence in the molecule of 2 atoms of radiobromine (Br^{82}), and the measurements which constitute the endpoints are carried out on a Geiger counter.

The bromo-dyes are colloidal in aqueous solution, as are their non-brominated counterparts. In protein-containing fluids, the dyes become associated with protein and if the protein is plasma protein, the dyes show a preferential affinity for albumin (12).[4] This "dye-protein bond" is not well understood but appears to be chemical rather than adsorptive in nature; it is quantitative in proportion (11, 12).

The amount of dye injected in these experiments varied from 15 to 30 mgm. per kgm. This dose is considerably higher than that ordinarily used for blood volume determinations with Evans blue because only extremely small amounts of dye normally appear in lymph early in the experiment, and it is necessary to give enough dye so that the early radioactive readings will be within the range of the Geiger counter. The serum concentration of dye at the start of the experiment is, therefore, in the range of 0.03 per cent to 0.05 per cent, and by the end of 3 hours has fallen to half this figure. This concentration is within the limits described by Rawson in which the dye is virtually entirely associated with albumin. The lymph concentration is, of course, much lower than this, and in the normal animal, the lymph from the leg or cervical lymphatic shows no gross color. The radioactive emanations, however, permit the detection of as little as 0.00025 mgm. of the dye.

The radioactive figures used in the experiments are expressed in arbitrary "units" which are derived as follows. The Geiger counter readings (in "counts per minute") are made on samples of 0.5 to 1.0 cc. of lymph or serum. These determinations are corrected for decay according to the 34-hour half-life of the isotope involved. This result is then expressed in thousandths of a micro-curie (so-called "milli-micro-curie," determined by reference to a uranium standard as described by Moore and Tobin (10)). The plasma concentration at 3 hours is then taken as a reference point and defined as 10 units per cc. Whatever correction factor is required to bring the 3-hour reading to a numerical value of 10.0 is then applied to the entire experiment.[5] Thus, a set of curves is obtained whose absolute level has been changed but whose relationship to each other is unaltered. Furthermore, the curves are readily comparable with other experiments in spite of the fact that the actual radioactive readings may have varied over a wide absolute range, due to the variation in strength of the original samples of radiobromine as they came from the cyclotron.

The protein determinations were made with the refractometer with frequent checks by Kjeldahl determinations. "Specific activity" of the protein, a ratio of the "active" to "inactive" protein present, is based on these protein readings and derived in a manner described in the following section.

[3] Such dyes have been shown by means of their radioactivity to collect in increasing concentration in areas of inflammation (10).

[4] The authors are indebted to Dr. Rawson for demonstrating that these brominated dyes behave much as their non-brominated precursors.

[5] For example, if, at 3 hours, the serum concentration is 5.0 m.μc. per cc., a factor of $\times$ 2.0 must be applied to make this value reach the arbitrary level of 10.0 "units" per cc. This factor of $\times$ 2.0 is then applied to all the figures in the experiment.

The animals used were dogs weighing from 15 to 25 kgm.; they were maintained under nembutal anesthesia, with the exception of a few in which control dye studies were made. Various lymphatic trunks were cannulated. The trunk of one or more legs was cannulated above the ankle joint, as described by Drinker (6). The cervical trunk on the left side, and the thoracic duct, were cannulated at a point near their entry into the left innominate vein. In addition to radioactivity, the rate of flow and the concentration of various chemical constituents of the lymph were measured before and after the burning of one or both hind feet to just above the ankle. Samples of arterial blood were removed at intervals from either the carotid or femoral arteries. The mean arterial blood pressure was recorded periodically with a mercury manometer by puncture with needle without interruption of the blood flow. The flow of lymph in the lymphatic trunks of the leg and neck is sluggish in the normal resting dog under nembutal anesthesia. In order to obtain sufficient lymph for analysis, it is usually necessary to stimulate the flow of the lymph from the foot by gentle massage of the foot pad. This massage is made to simulate the pressure on the pad of walking. The flow of lymph in the cervical trunk is stimulated by manually flexing the head on the neck (14), approximately 15 times per minute. Following burning of the leg, no massage or pressure was exerted.

The flow of lymph in the thoracic duct is so vigorous under normal conditions that the cannula has to be stoppered when samples are not being removed. The flow in the thoracic duct is generally maintained, even when the animal has a low blood pressure late in the shock state, but occasionally spontaneous flow disappears. It can then be produced readily by pressure on the abdomen. The method employed in cannulating the thoracic duct cuts off the normal flow from the duct into the vein. It is probable, though it was not demonstrated in these experiments, that the flow of lymph normally carried through the thoracic duct is continued to some extent through collaterals with the right thoracic lymphatic.

EXPERIMENTS

1. *Rate of formation of lymph*

Little is known regarding the rate of passage of water and electrolytes from blood stream to

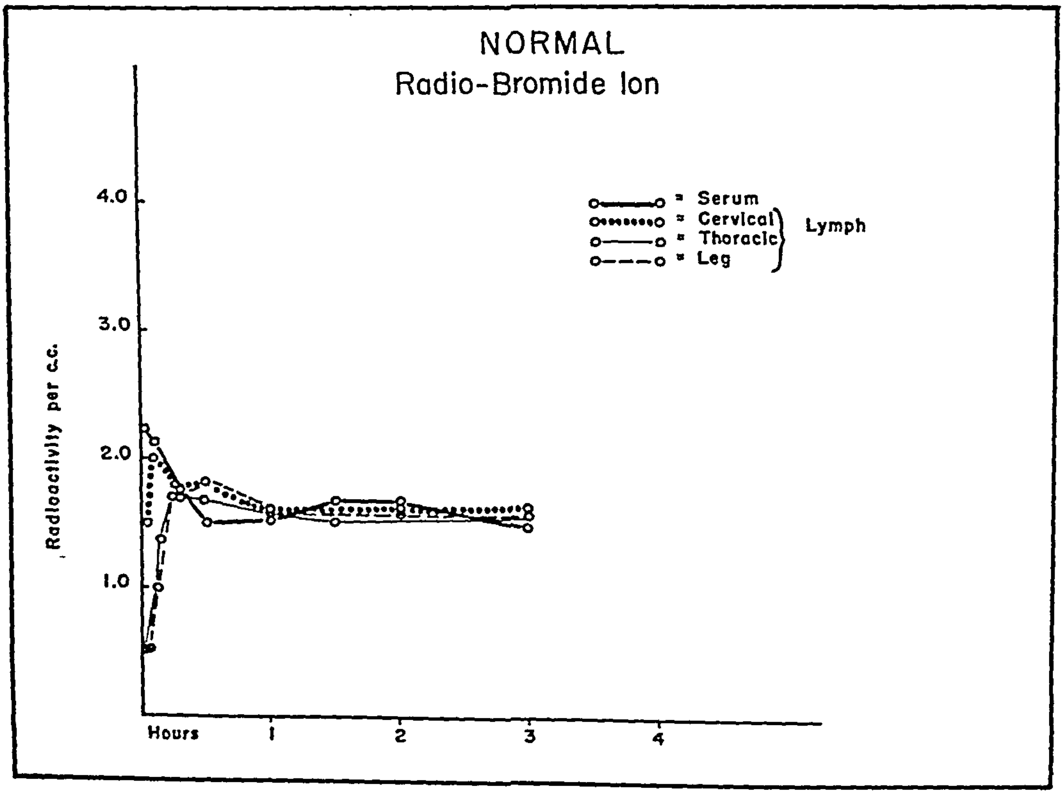


Fig. 1. Rate of Distribution in Blood and Lymph of Radioactive Bromide Ion in a Control Dog Under Nembutal

The radioactive bromide (Br^{82}), as the sodium salt, was injected intravenously at 0 hours. Samples of blood serum, cervical, thoracic, and leg lymph were collected at 5-minute intervals during the first 20 minutes and at each half hour through the first 2 hours. Equal concentrations of radioactivity were reached in the various fluids at 20 minutes.

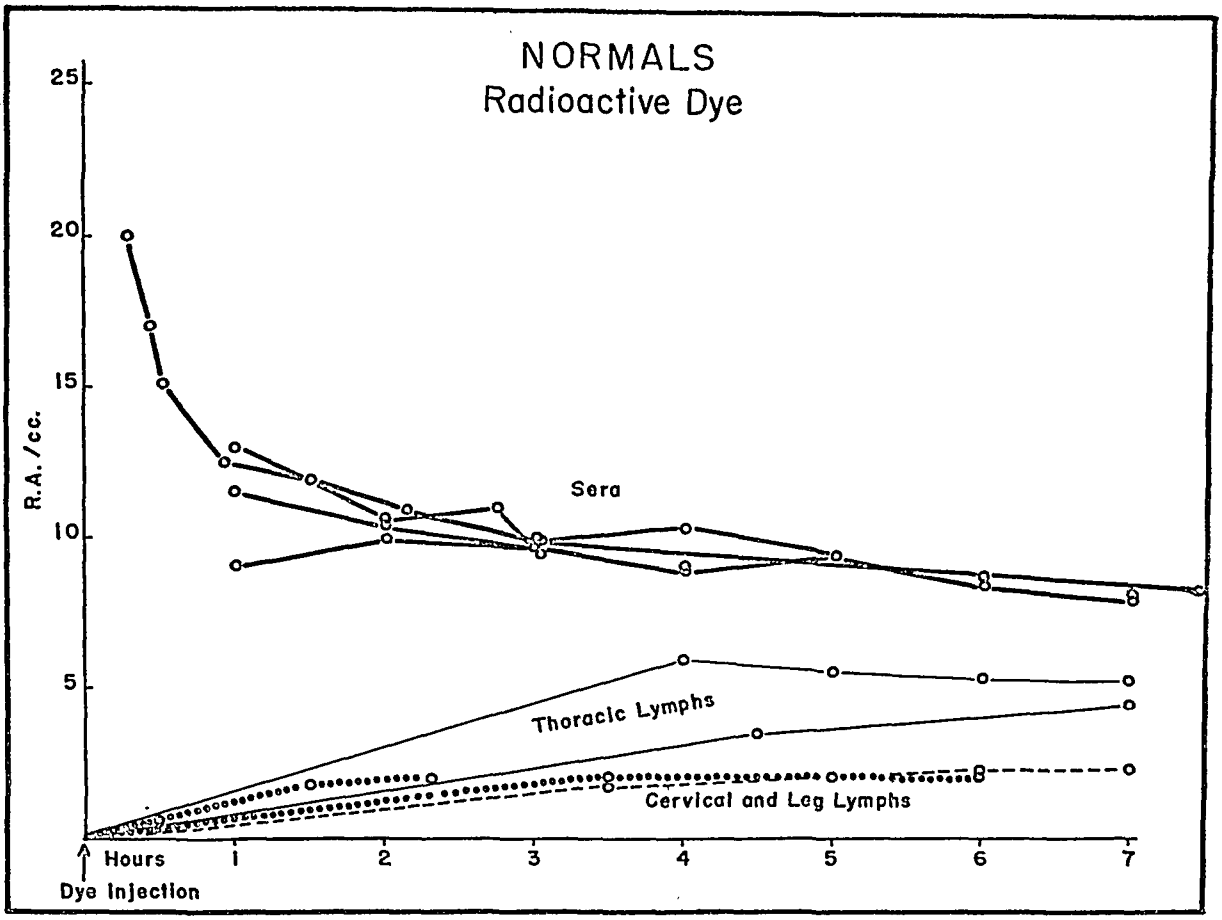


FIG. 2. THE CONCENTRATION OF RADIOACTIVITY IN BLOOD SERUM, THORACIC, CERVICAL, AND LEG LYMPH FOLLOWING THE INTRAVENOUS INJECTION OF RADIOACTIVE DYE IN 4 NORMAL DOGS, 1 UNDER NOVOCAINE AND 3 UNDER NEMBUTAL ANESTHESIA

The rate of equilibration following injection of dye is slower than after injection of the bromide ion. The concentration in lymphs does not become constant until after the second hour; equality with serum is not reached.

lymphatic vessel and of the exact relation of lymph to interstitial fluid. Drinker and his collaborators have developed the concept that the fluid in a lymphatic trunk is essentially interstitial fluid since no anatomic structure which might act as a semi-permeable membrane has been demonstrated at the entrance to the lymphatic vessels. It is possible, however, that water is reabsorbed into the blood stream at the venous end of the capillary from the interstitial fluid just as this fluid enters the collecting lymphatic. This would result in an increased concentration in the lymph, as compared to the interstitial fluid, of those substances, such as protein, which pass only slowly through the capillary membrane. The more rapidly a substance injected into the blood stream can be identified in the lymphatic, the closer the lymphatics are functionally to the capillary membrane, and the more comparable lymph should be to interstitial fluid.

In order to determine how rapidly an ion, readily permeable through the capillary membrane, comes into equilibrium in the body fluids, radioactive bromide ion, as the sodium salt, was injected intravenously. The concentrations of the radioactivity were followed in the blood stream and in the leg, cervical, and thoracic lymph at 5-minute intervals. The results are shown in Figure 1. A rapid fall in the concentration of radioactivity was found in the blood stream, and a rapid rise in all the lymphs. At 20 minutes, the radioactivity of the 4 fluids was equal, that is, equilibrium had been reached, and continued equilibrium was observed throughout the 3 hours of the experiment. This demonstrates graphically the permeability of normal capillaries to a small inorganic ion and is to be

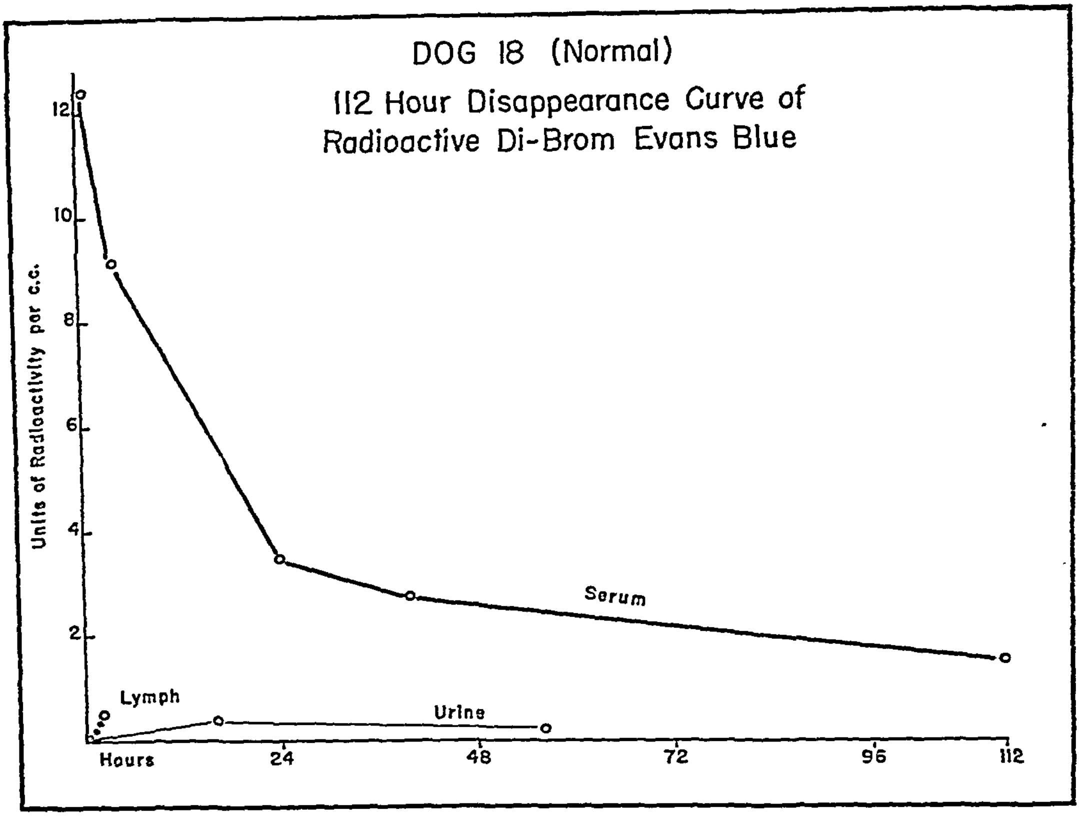


FIG. 3. ONE HUNDRED AND TWELVE HOUR DISAPPEARANCE CURVE OF RADIOACTIVE EVANS BLUE DYE IN THE BLOOD SERUM OF A NORMAL DOG

contrasted to their impermeability to the large organic colloidal molecules, demonstrated in the succeeding sections. In certain pathologic conditions, this distinction is virtually lost, and the capillary becomes almost as permeable to the colloids as it formerly was only to ions.

The appearance of significant amounts of radioactive bromide ion in the lymphs from the 3 areas in so short a period and the rapid achievement of equilibrium between blood and lymph demonstrates the physiologic proximity of fluid within the lymphatic trunk to interstitial fluid and the capillary membrane. It is to be emphasized that in this experiment, nembutal anesthesia was used, and there was no trauma except that incident to the cannulation of the lymphatics. No burn was performed.

2. Normal distribution of radioactive proteins

In order to learn the normal distribution of the dye-protein molecule between blood and lymph from various areas, radioactive dye was injected into 13 dogs which were not burned. In 10, the lymphatic cannulations and sample collections were made under local anesthesia (1 per cent procaine). The other 3 were maintained under nembutal. No difference in dye-protein distribution was noted between the 2 groups.[6] The results in 4 of the experiments are shown in Figure 2.

The important finding in these normal animals is that the lymph and blood curves form a characteristic pattern, rendering the normal state of capillary permeability readily definable by this technic. Although there are minor variations between animals, there are limits of normal to the concentration of the radioactive dye in the lymph. The serum disappearance curve is a slope which falls at a constantly decreasing rate.[7]

[6] *Since these experiments were concluded, Beecher and his collaborators (15) have shown that nembutal decreases the flow of lymph. Our experiments are consistent with this finding.*

[7] The disappearance of radioactivity in the serum in the

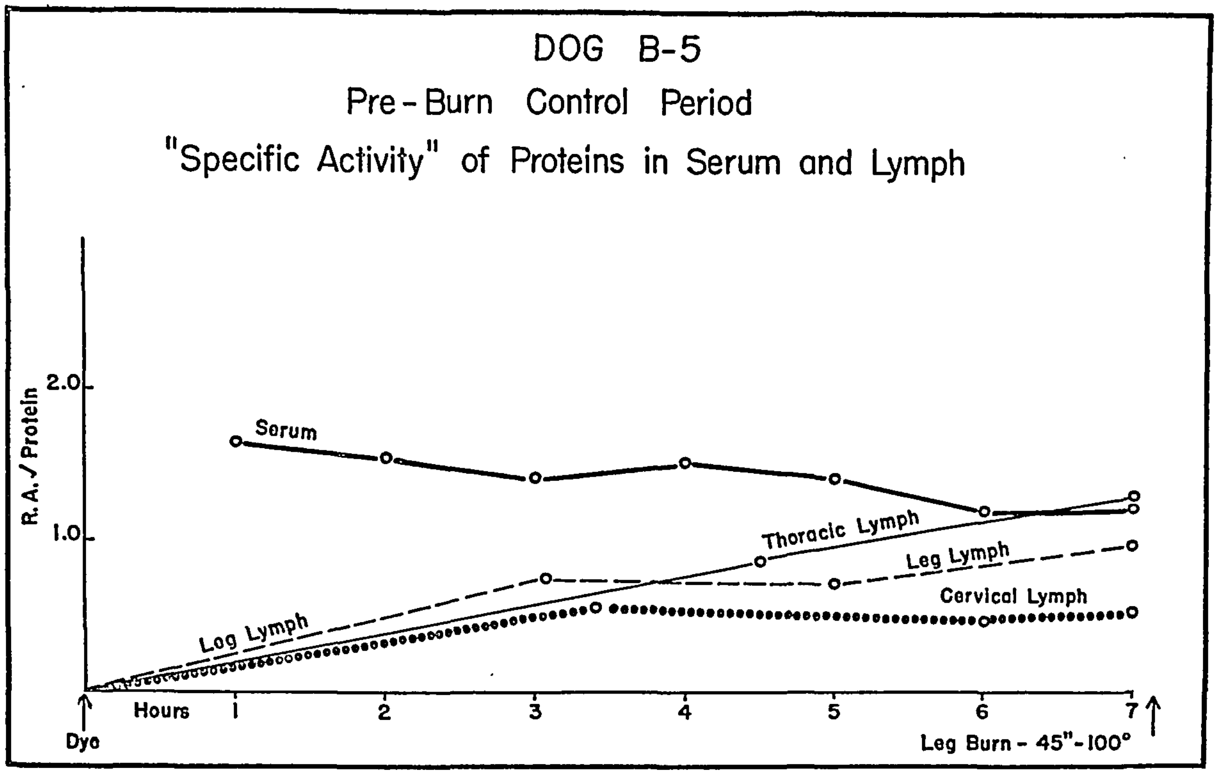


FIG. 4. "SPECIFIC ACTIVITY" OF PROTEINS IN SERUM AND LYMPH FOLLOWING INTRAVENOUS INJECTION OF RADIOACTIVE DYE IN AN UNBURNED DOG UNDER NEMBUTAL

$$\text{“Specific activity”} = \frac{\text{Radioactivity in units per cc.}}{\text{Protein in grams per 100 cc.}}.$$

Specific activity of the thoracic lymph reaches that of the serum between the sixth and seventh hour, indicating that thoracic lymph is in equilibrium with serum at that time. At the seventh hour, the specific activity of neither leg nor cervical lymph has reached that of the serum.

The concentration of radioactivity in peripheral lymph (cervical and leg) tends to fall in the range of 1.0 to 2.0 units per cc. Any value above 2.5 units per cc. (a 25 per cent increase over normal) is regarded as pathologic. The concentration in thoracic lymph rarely reaches a 3-hour plateau higher than 5 units per cc. in the normal animal.

The disappearance curve of the dye in the blood serum was followed in 1 normal animal for 112 hours (Figure 3). The colloidal dye is very slow to leave the blood stream completely and, in fact, at 112 hours is still at about one-sixth of its 1-hour value. The slope is one of decrease at a constantly decreasing rate.[8]

first hour was measured in only 1 animal of this group as we have not found its steep slope significant for our purposes; after the 1-hour reading, the values tend to coincide.

[8] The radioactivity recovered in the urine and lymph in the first 56 hours of this experiment accounted for but a small portion of that disappearing from the blood stream. Other evidence (13) has shown that a large part of the dye is slowly excreted in the bile and feces.

Since the dye is associated with protein in the plasma, it is of interest to determine the ratio of radioactive dye to total protein in serum and lymph. This will indicate to what extent equilibrium has been approached in the exchange of dye and protein across the capillary. If the ratios in serum and lymph are equal, then no matter what the absolute values of the various components are, equilibrium has been reached.

Assuming that the dye is "bound" to protein, this ratio of dye to protein may be termed the specific activity of the protein, that is, the amount of radioactivity per unit protein, and in general terms:

$$\text{Specific activity of the protein} = \frac{\text{Radioactivity per unit volume}}{\text{Protein per unit volume}}$$

Using such an expression, if the specific activi-

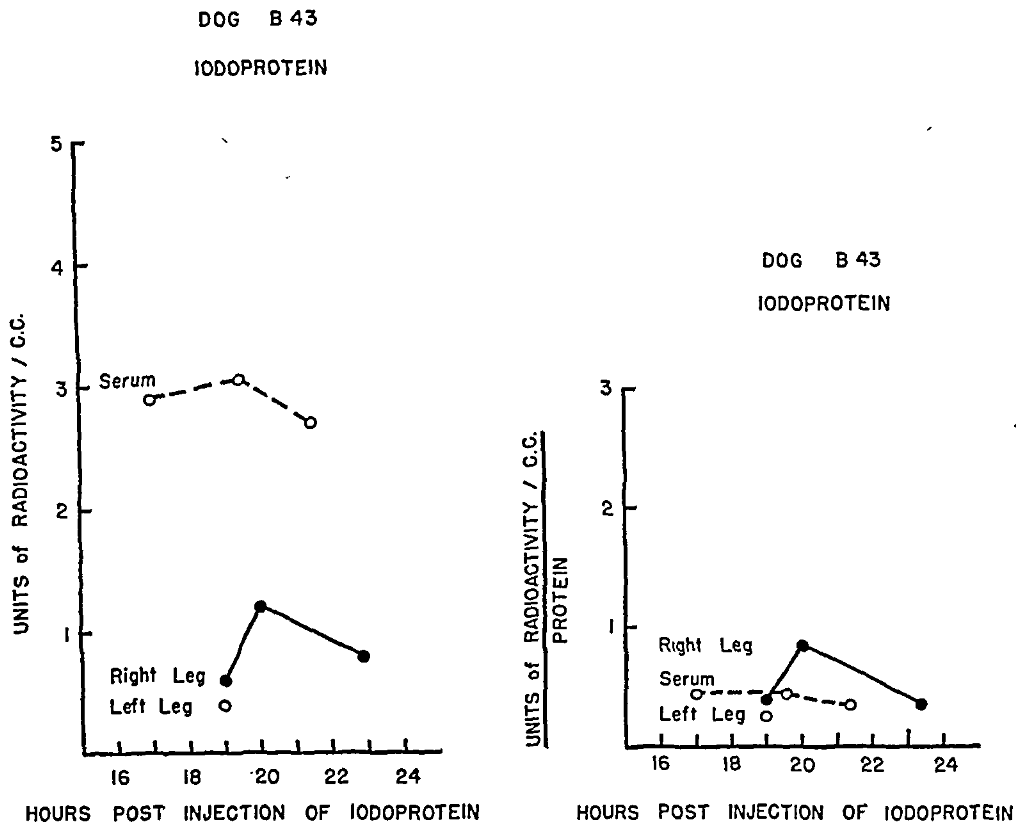


FIG. 5. RADIOACTIVITY IN BLOOD SERUM AND LYMPH FROM BOTH HIND LEGS OF AN UNBURNED DOG UNDER NEMBUTAL, 17 TO 23 HOURS AFTER THE INTRAVENOUS INJECTION OF RADIOACTIVE IODOPROTEIN

(a) *Concentrations of radioactivity.* Serum and lymph have not reached equality.

(b) *Specific activity* (radioactivity per unit of protein). This calculation suggests that the peripheral lymph has reached, by this time, equilibrium with the serum.

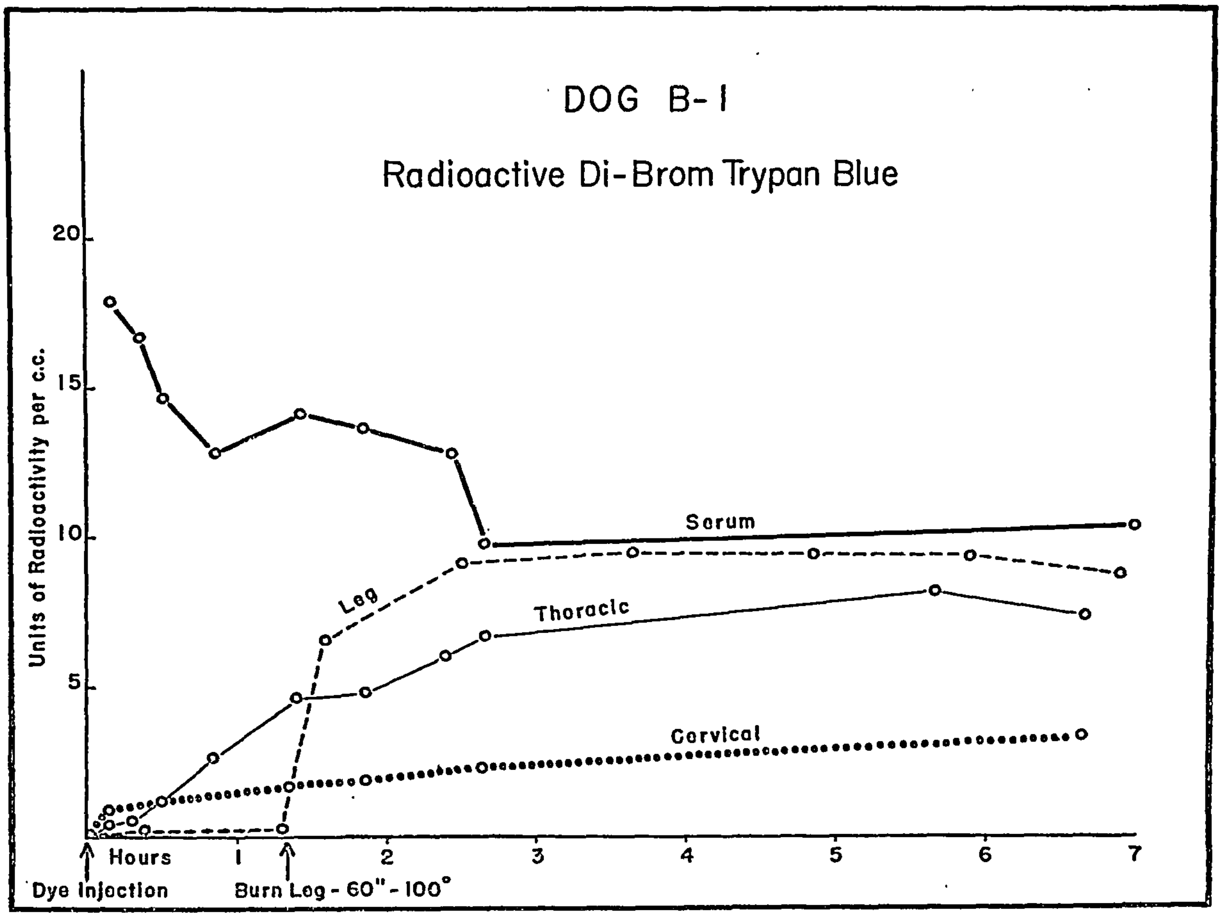
ties on the two sides of the capillary are the same, equilibration has taken place and exchange of dye-protein has reached a point beyond which it cannot theoretically progress so long as the dye molecules stay attached to protein molecules, and so long as we are dealing with a passive membrane which does not exert any force to exclude dye from one side or the other.

The validity of these theoretical considerations is borne out by the fact that when specific activities are computed for serum and lymph, we do indeed find that, in certain pathologic conditions, exchange reaches equilibrium, whereas in the control animal, where permeability to these colloids should be limited, such equilibrium is not reached save in the case of thoracic lymph. As is well known (16), the protein concentration of thoracic lymph is considerably higher than that of peripheral lymph, and in its content of albumin approaches that of plasma.[9]

The results of the calculation of specific activity in terms of the total protein are shown in Figure 4, which is to be contrasted with Figure 2. In Figure 2, the concentration of radioactive units per cc. is shown. In Figure 4, the specific activity of the proteins present has been computed according to the formula:

$$\text{Specific activity} = \frac{\text{Radioactivity in units per cc.}}{\text{Protein in grams per 100 cc.}}$$

[9] Calculation of the specific activity of the protein is complicated by two factors: one, that the dye is preferentially bound to albumin (12), and the other that the albumin-globulin ratio in lymph may be raised following a burn, as mentioned later. We have not measured these variables quantitatively and have found the calculation presented above satisfactory for our present purposes.



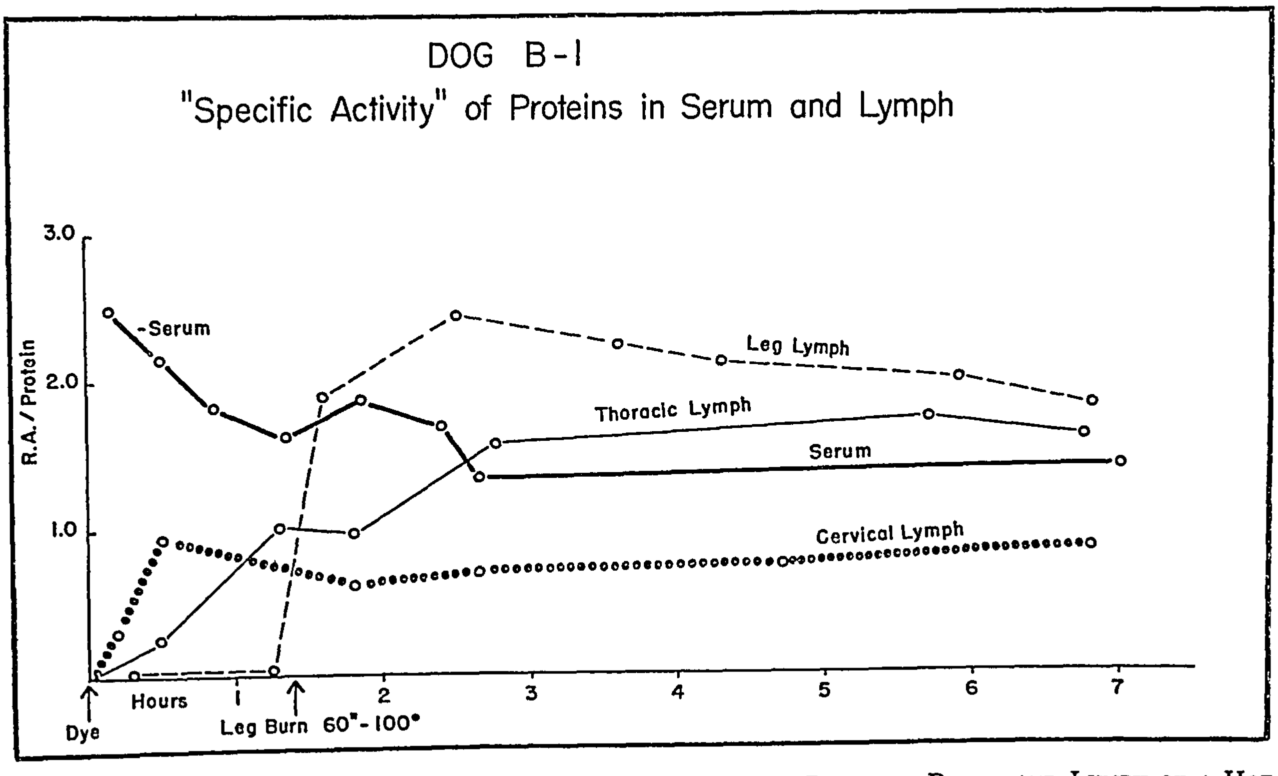


FIG. 6. THE EFFECT ON THE DISTRIBUTION OF RADIOACTIVE DYE BETWEEN BLOOD AND LYMPH OF A HOT WATER BURN OF THE LEG OF A DOG UNDER NEMBUTAL

The burn was severe, boiling water for 60 seconds.

(a) *Concentration of radioactivity.* Immediately following the burn, there is an abrupt rise in concentration in the lymph from the burned leg which reaches that of the blood serum within 2 hours. In the blood serum,

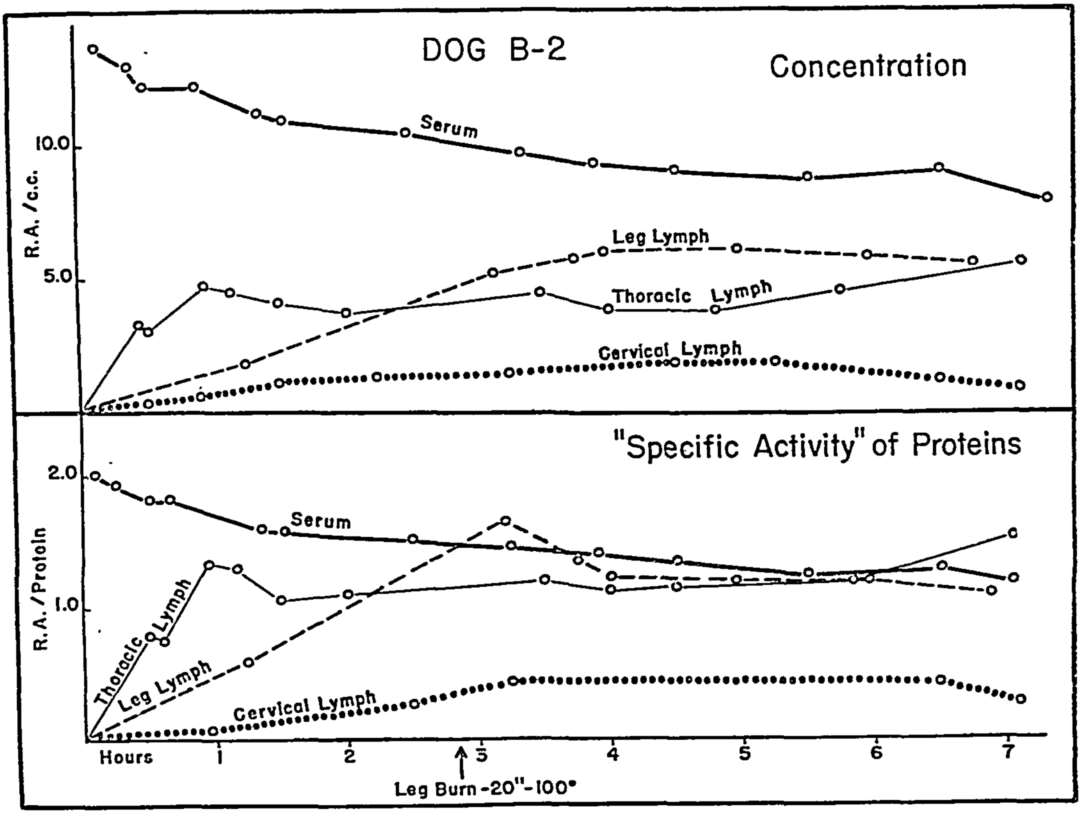


FIG. 7. CONCENTRATIONS OF RADIOACTIVE DI-BROM EVANS BLUE AND SPECIFIC ACTIVITY OF PROTEIN IN BLOOD SERUM AND LYMPH IN A DOG UNDER NEMBUTAL BEFORE AND AFTER THE BURN OF A HIND LEG WITH HOT WATER

The burn was less severe than that in the experiment of Figure 6, boiling water for 20 seconds. The concentration of radioactivity in the lymph from the burned area did not reach that of the blood serum; the specific activity was equal to but not greater than that of the serum after the burn for the 4 hours before sacrifice of the dog.

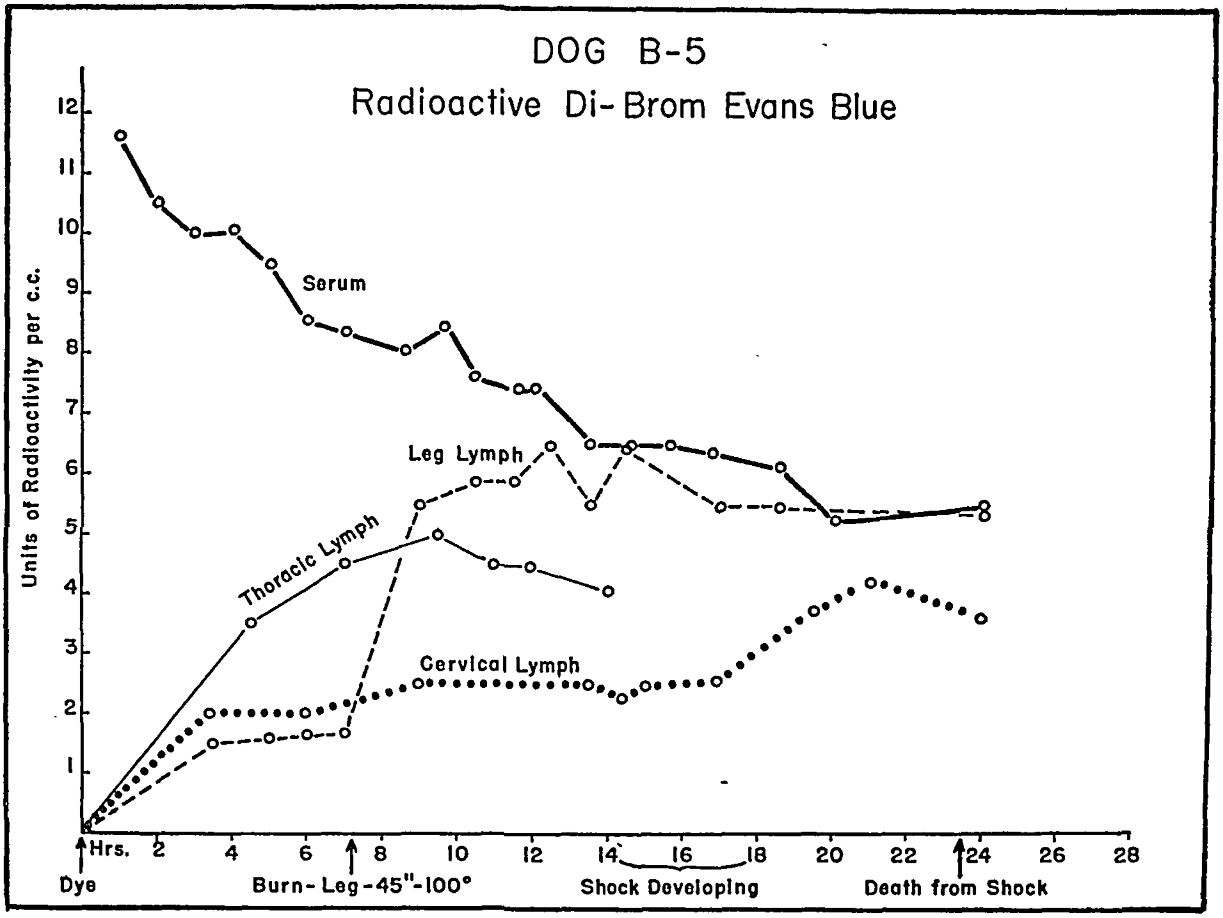
It will be noted that in the normal animal, thoracic lymph reaches equilibrium with serum (the curves come together) in about 6 hours, whereas the peripheral lymph does not reach equilibrium at this time. This is in contrast to the pathologic conditions described below.

In Figure 5 a and b, are shown the radioactivity and specific activity in serum and leg lymph of a radioactive iodoprotein, 17 to 23 hours after its injection into the blood stream. After this long interval, equilibration has been reached between the 2 fluids. The radioactive iodoprotein [10] used in this experiment has the advantage of a longer half life, 8 days as compared with 34 hours for radioactive bromine.

[10] The iodoprotein and the bromoprotein, used in the experiment shown in Figure 11, were kindly prepared for us by Dr. Arnold M. Seligman. Radioactive iodine and bromine were conjugated with dog serum proteins *in vitro*, as described by Fine and Seligman (5).

immediately following the burn, there is a slight rise in concentration of radioactivity; such an interruption in the smooth slope of the serum curve immediately following the burn has been encountered in several experiments. The thoracic or cervical lymphs for the 7 hours before the animal was sacrificed showed no departure from normal.

(b) *Specific activity.* This radioactivity-protein ratio was higher in the lymph from the burned leg than in the blood serum. This calculation suggests that the radioactive dye is bound preferentially to a portion of the serum protein which escapes in the burned area in greater proportion. The activity of thoracic lymph reaches that of the blood stream at 2½ hours while the cervical lymph from a non-burned area does not reach equilibrium in the 7 hours of the experiment.


DOG B-5
Radioactive Di-Brom Evans Blue
Units of Radioactivity per c.c.
Serum
Leg Lymph
Thoracic Lymph
Cervical Lymph
Hrs.
Dye
Burn-Leg-45"-100°
Shock Developing
Death from Shock

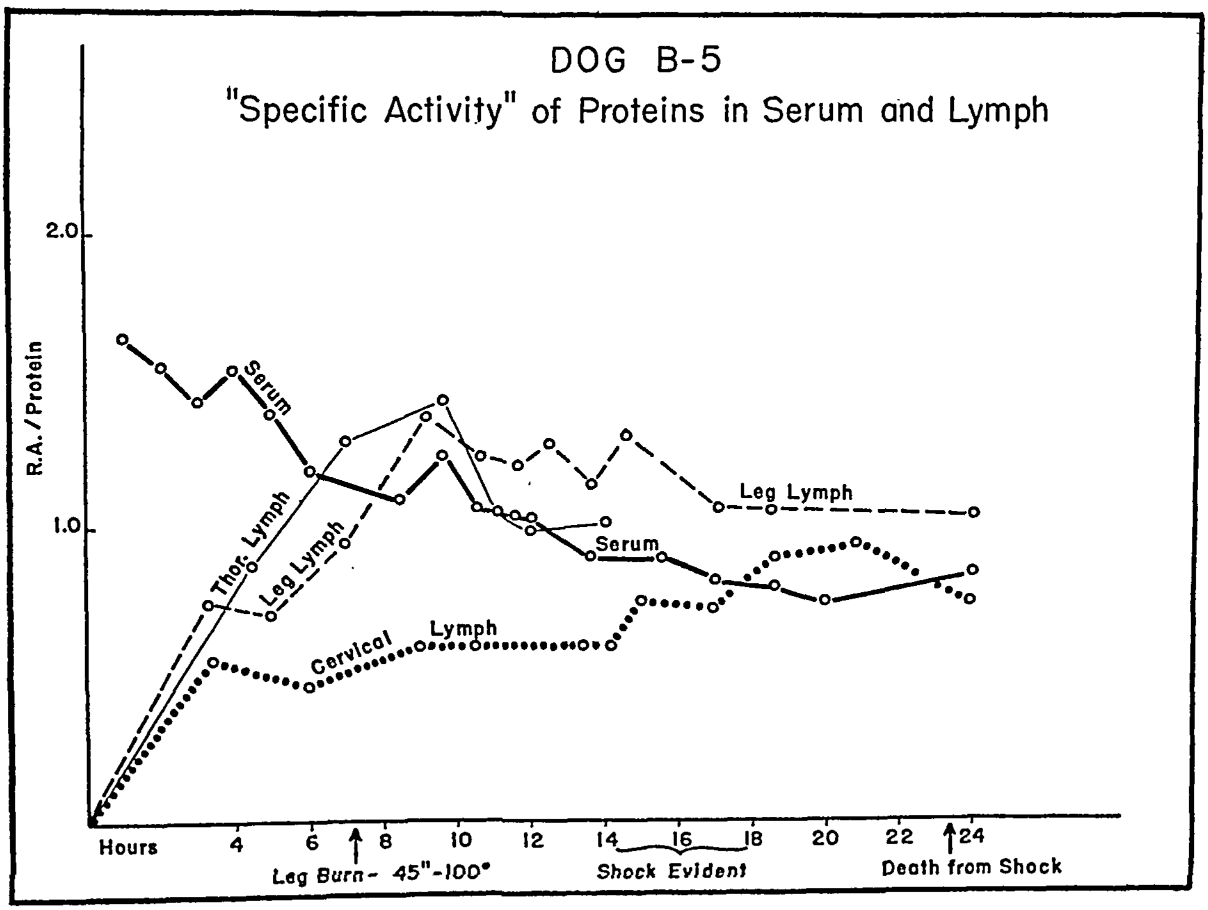

DOG B-5
"Specific Activity" of Proteins in Serum and Lymph
R.A./Protein
Serum
Thor. Lymph
Leg Lymph
Cervical Lymph
Hours
Leg Burn- 45"-100°
Shock Evident
Death from Shock

FIG. 8.

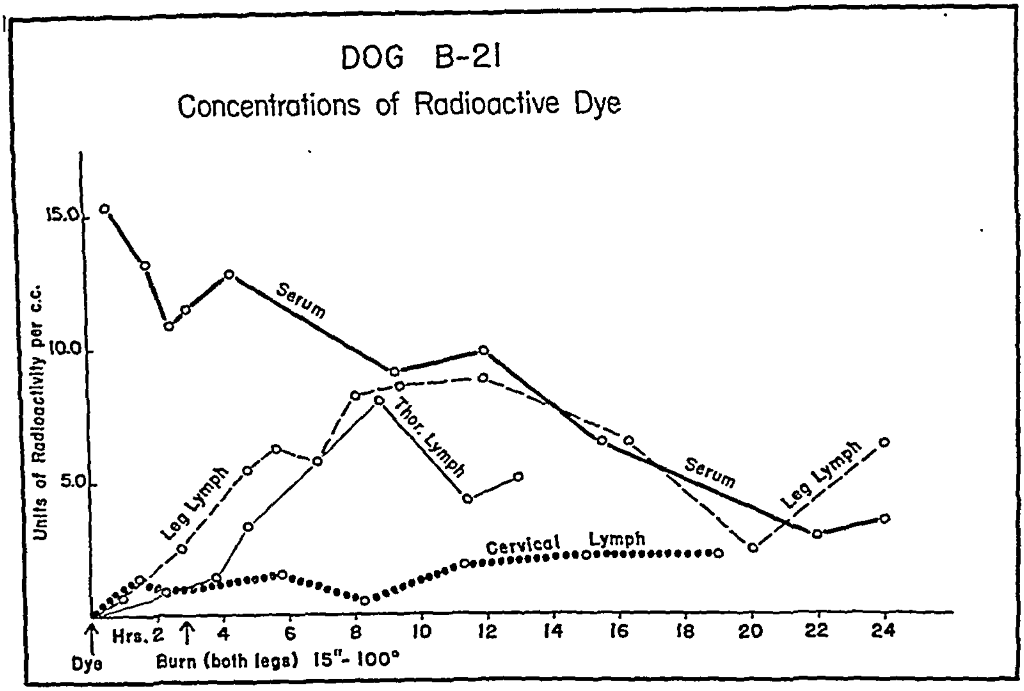


FIG. 9. CONCENTRATIONS OF RADIOACTIVE DYE IN BLOOD SERUM AND LYMPHS IN A DOG UNDER NEMBUTAL FOLLOWED UNTIL DEATH IN BURN SHOCK

Both hind legs were burned in boiling water for 15 seconds. The concentration of radioactivity in the lymph from the burned area reached that of the blood stream more slowly than in the experiment with a more severe burn (Figure 6). The flow of lymph from the non-burned area, drained by the cervical trunk, ceased after the animal had been in shock for a few hours; while lymph was still flowing, no change occurred to suggest a change in capillary permeability.

That radioactive halogenated proteins are treated similarly to the dye proteins by the capillary membrane is suggested by the observation with bromoprotein, shown in Figure 11, and described in the succeeding section.

3. Effect of burns on capillary permeability

As anticipated from the work of Drinker and his collaborators (6, 7), the flow and protein concentration of lymph is increased from the burned leg of a dog. After immersion of the foot and ankle in boiling water, the flow from the lymphatic trunk just above the burned area rises many-fold, and the protein concentration rises from 2 to 2.5 grams, to 4.5 to 5.0 grams per 100 cc.[11] The flow rapidly reaches a maximum, usually within the first half hour, and slowly falls off during the next 5 hours, as edema of the burned area increases. When maximum edema of the burned area has been reached, the flow has descended to a low plateau, above the pre-burn flow, at which rate it remains until either the blood pressure falls to a low level or the swelling rises above the burn to the cannula, shutting off the lymphatic trunk. The rise in protein concentration is sustained.

[11] The effect of temperature and duration of the burn on lymph flow and protein concentration will be detailed in a subsequent paper from this laboratory (17).

FIG. 8. THE RADIOACTIVITY OF THE BLOOD SERUM AND LYMPHS OF A DOG UNDER NEMBUTAL OBSERVED UNTIL DEATH IN BURN SHOCK

Both hind legs were severely burned, boiling water for 45 seconds. Changes occurred in lymph from the burned area similar to those shown in Figure 6. Following the development of low blood pressure shock, there was evidence of increased permeability in the non-burned areas of the head drained by the cervical lymphatic trunk.

(a) *Concentration of radioactivity.*
(b) *Specific activity.*

When radioactive dye has been injected into the dog previous to immersion of the foot in hot water, there is a prompt rise in radioactivity immediately following the burn as might be expected from the rise in protein concentration. If the burn has been severe (boiling water for 15 seconds or longer), the concentration of radioactivity of the lymph from the burned area rapidly reaches that of the blood serum. When this happens, the specific activity of the lymph will be above that of the serum, since the total serum protein averages 2 grams more than the lymph protein, even after burning. In Figure 6 a and b, are depicted the findings in a dog of which a leg was immersed in boiling water for 60 seconds. There is no deviation from the normal in the radioactivity concentration in the lymph from the cervical and thoracic unburned areas for the 6 hours before the dog was sacrificed. Two similar short experiments produced comparable but not identical results; the data of one are shown in Figure 7, in which the burn was not as severe and the effect upon the capillary premeability less (see legend).

The only possible explanation for the equality of radioactivity in lymph and serum and for the greater specific activity of lymph following a severe burn is that the radioactive dye is preferentially attached to one portion of the serum protein and that this portion exudes through the damaged capillaries in greater proportion. This is indirect, confirmatory evidence of the finding of Rawson (12), that the Evans and trypan blue dyes bind themselves to the albumin of the serum.[12]

In 5 experiments, the blood and lymph of dogs with burned legs were observed until death in shock from 17 to 27 hours after burning. These

[12] In other dogs similarly burned, the albumin-globulin ratio, determined by precipitation methods, of the post-burn lymph is, in general, increased and is greater than that of the serum.

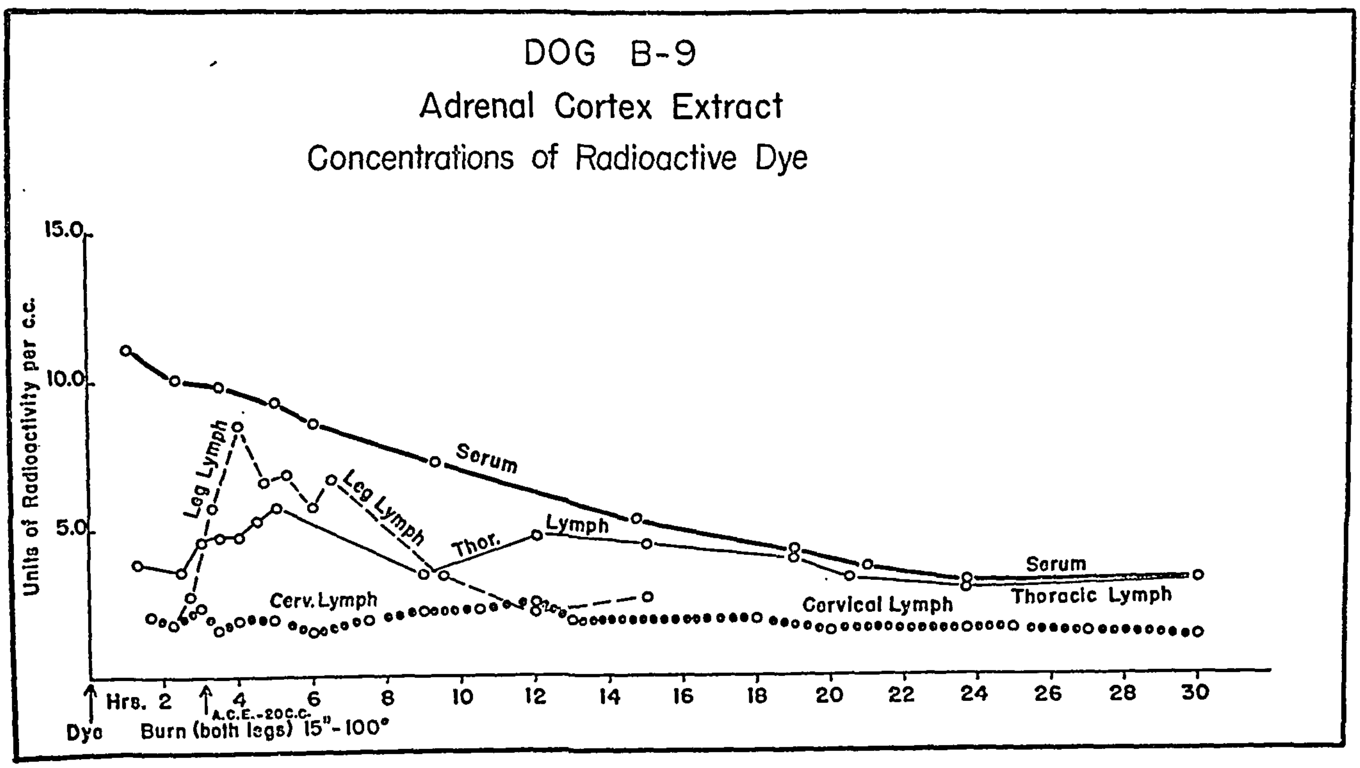


FIG. 10. CONCENTRATION OF RADIOACTIVE DYE IN BLOOD SERUM AND LYMPHS IN A DOG BURNED, GIVEN ADRENAL CORTICAL EXTRACT INTRAVENOUSLY, AND FOLLOWED UNTIL DEATH IN SHOCK

Extract, 20 cc., was given immediately following a burn of both hind legs in boiling water for 15 seconds. The concentration of radioactivity of the lymph from the burned area rose precipitously but remained at a level below that of the serum for the 12 hours during which the flow continued. This observation is not considered significantly different from those in other experiments where no extract was injected. No deviation in the course of the concentration of the radioactivity in the serum, thoracic, and cervical lymph from the other experiments was noted. There was no evidence of increased capillary permeability in the non-burned region at any time before death.

experiments of long duration were planned to obtain evidence regarding possible changes in capillary permeability in areas of the body remote from the burn, in contrast to the experiments of short duration, in the first part of this section, in which local changes only were found. In 4 of the 5 dogs, both hind legs were immersed in hot water to increase the area burned and hasten the onset of shock. All of the animals died following a period of low blood pressure with hemoconcentration and other findings in the blood typical of shock from burns. In only one, the first experiment, was evidence found in an area remote from the burn of increased capillary permeability which could be ascribed to shock. The data of this experiment are shown in Figure 8 a and b. From the fourteenth to the eighteenth hours, the mean arterial pressure gradually fell to a level below 80 mm. Hg. From the twentieth to the twenty-fourth hours, the lymph from the cervical trunk showed an increased concentration of radioactivity, consistent with an increase in permeability. This increase is approximately 25 per cent above normal, an order of magnitude considered significant by this technic. There was no evidence of infection in either the upper respiratory passages or the cannulation wound which could account for the increase in permeability (see section 5). The findings in the lymph from the burned area were identical with those of the short experiments. The thoracic duct

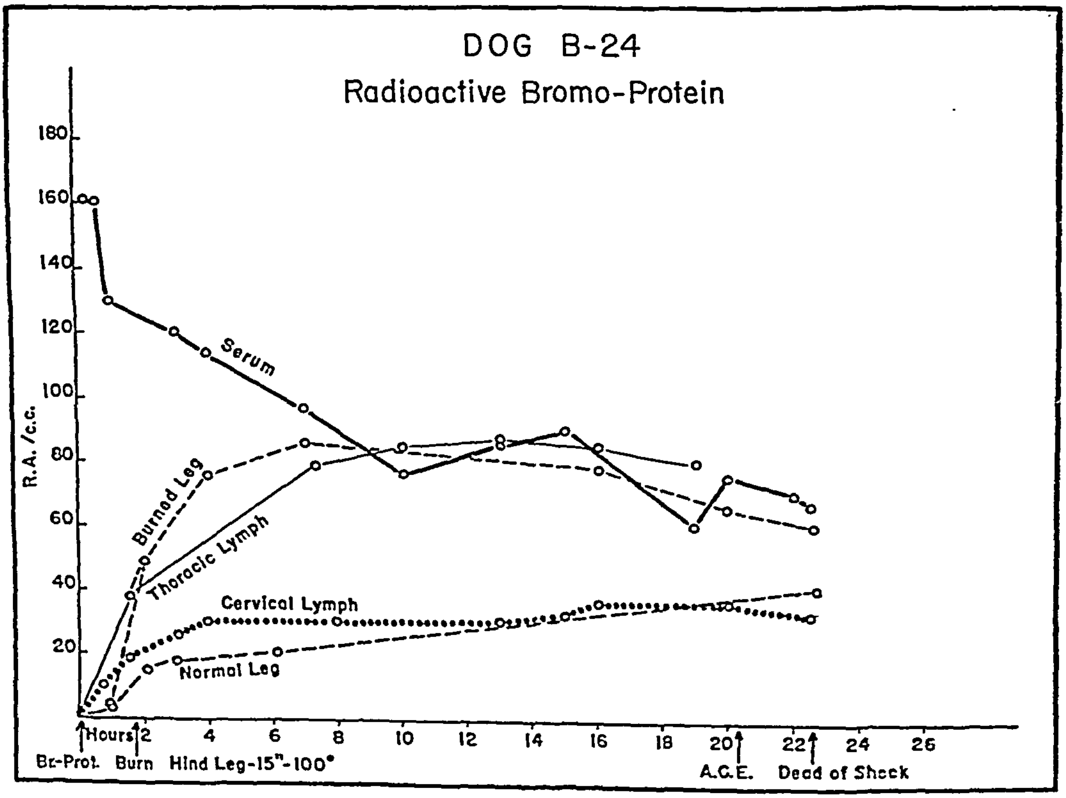


FIG. 11. CONCENTRATION OF RADIOACTIVITY IN BLOOD SERUM AND LYMPHS FOLLOWING THE INJECTION OF RADIOACTIVE BROMOPROTEIN IN A DOG UNDER NEMBUTAL, BURNED, AND GIVEN ADRENAL CORTICAL EXTRACT IN THE LATE STAGE OF SHOCK

One hind leg was burned in boiling water for 15 seconds; the expected rise in radioactivity occurred in the lymph from this area. There was no deviation in radioactivity in the lymph from the non-burned leg or head from that expected in a control dog. There was, therefore, no indication of any change in capillary permeability in the non-burned areas. Two hours before death, when the dog was in severe shock, 30 cc. of adrenal cortical extract was injected intravenously without any apparent effect.

cannula became plugged before the development of shock.

In Figure 9 are shown the data of the second experiment in which no change in permeability was demonstrated in the cervical lymph from a non-burned area. The animal developed shock associated with low blood pressure and hemoconcentration. Flow in the cervical lymphatic trunk ceased 4 hours before death, presumably because of continued diminished blood flow; the last sample of cervical lymph was collected when the animal was already in profound shock. The expected changes in concentration and flow were demonstrated in the lymph from the burned leg.

The other 3 experiments of long duration are described in the next 2 sections.

4. *Effect of adrenal cortical extract on capillary permeability induced by burns*

Adrenal cortical extract has been recommended as a therapy in burn shock (18, 19). It is probable that the adrenal cortex exerts an influence on capillary permeability in adrenal cortical insufficiency (20). In 3 of the long duration experiments, adrenal cortical extract was administered.[13] In 2, the extract was given immediately following the burn (Figures 10 and 12), and in 1, late in shock, 19 hours after the burn and 2 hours before death (Figure 11). The extract neither prevented nor reversed the changes in capillary

[13] We are indebted to The Upjohn Company for generous supplies of a potent extract of the adrenal cortex.

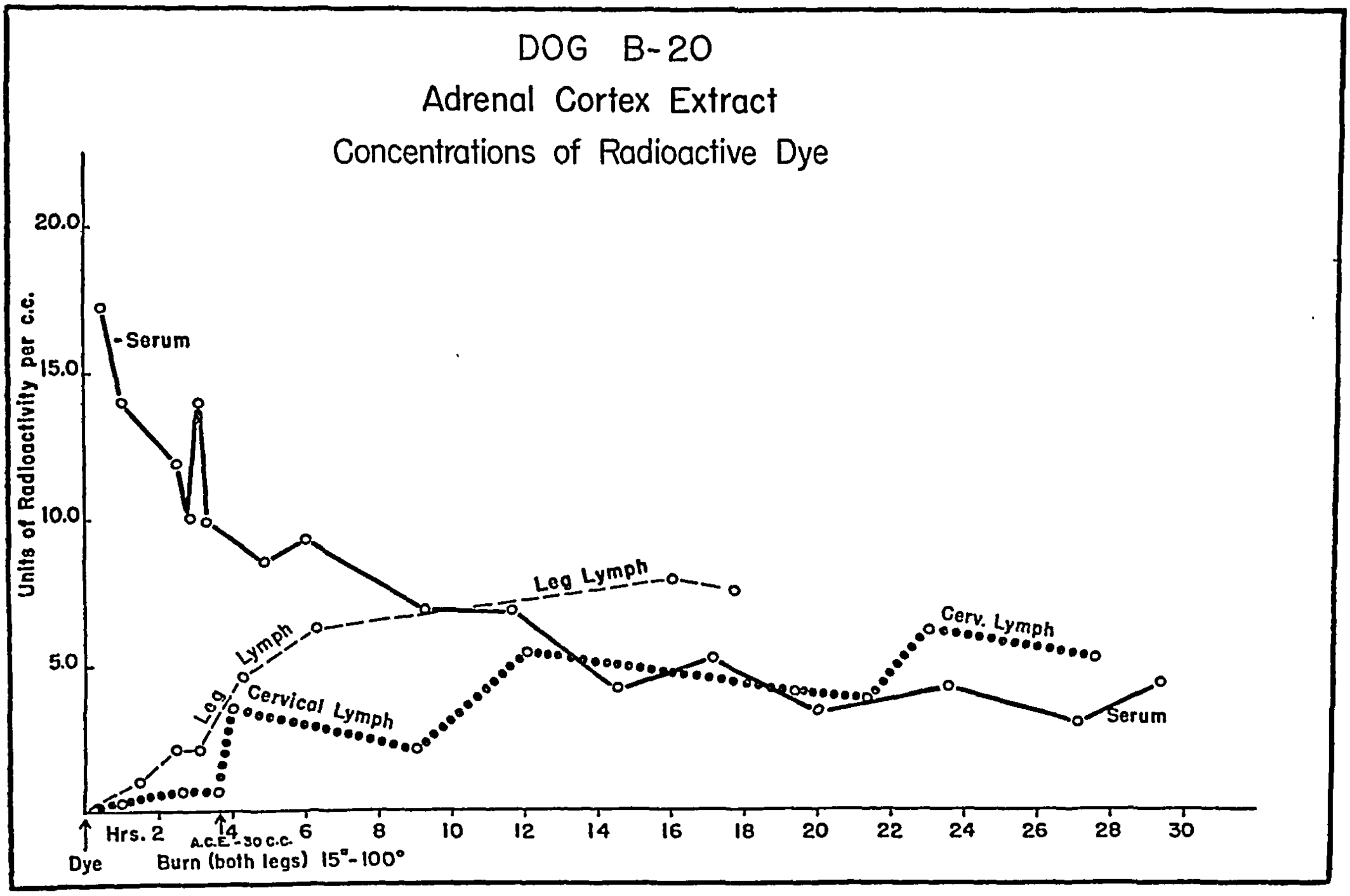


Fig. 12. Concentrations of Radioactivity in Blood Serum and Lymphs in an Animal Burned, Given Adrenal Cortical Extract, and Dying with Evidence of Infection in Head and Neck

Both hind legs were burned in boiling water for 15 seconds. Adrenal cortical extract, 30 cc., was injected immediately following the burn. The concentration of radioactivity in the lymph from the burned area rose abruptly and eventually more than equalled that of the blood serum in a manner comparable to the experiments in which no extract was given. A rise in concentration was observed in the cervical lymph 4 hours after the onset of the experiment which continued slowly until 7 hours before death when the concentration was higher than that of the blood serum. Post mortem examination showed a purulent infection of nasopharynx, mouth, trachea, and cervical cannulation wound. It is considered that the infection accounts for the alterations in capillary permeability, indicated by the increased concentration of radioactivity in the cervical lymph.

permeability in the burned area, and it did not influence the permeability in the non-burned regions.

5. *Infection and capillary permeability*

The experiment in Figure 12 illustrates a change in permeability which is the result of a complicating infectious process rather than of the burn. It is probable that the dog had an antecedent infection of the upper respiratory passages. The experiment warns against the unwitting use of an infected animal and emphasizes the necessity of maintaining strict aseptic precautions throughout the surgical procedures of lymphatic cannulation in such experiments.

An alteration in permeability is demonstrated in that portion of the head drained by the cervical lymphatic trunk in addition to that encountered in the burned leg. This change in the non-burned area was unexpected because it occurred soon after the burn and onset of the experiment, and long before the animal showed manifestations of shock. The altered permeability was maintained throughout the remaining 24 hours of the experiment, and at the thirteenth hour, the radioactivity in the cervical lymph equalled that of the blood. At post mortem examination, the mouth, nasopharynx, and trachea were filled with greenish, purulent exudate, and the cervical cannula wound was grossly infected. There was undoubtedly an acute inflammatory process in the region from which the lymph was flowing into the cervical lymphatic trunk. It is believed that this process was responsible for the demonstrated change in capillary permeability.

COMMENT

The observations on the distribution of radioactive molecules between blood and lymph recorded in this paper add to the understanding of certain physiologic mechanisms of the normal circulation, as well as of the abnormal, induced by burns. A rapid exchange of electrolyte and a slower exchange of protein through the interstitial space is recorded. A considerable proportion of the plasma protein circulates outside of the blood stream.

The Evans (T-1824) and trypan blue dyes have been widely used to measure plasma volume and there has been disagreement regarding the correct interpretation of the falling concentration of dye in the blood stream after injection. The experiments in this paper show that the dyes measure not plasma volume but truly the fraction of the plasma protein with which they combine, whether this protein remains in the blood stream or escapes into the interstitial spaces. The observations do not invalidate the dye method of estimation of plasma volume but lead to a different interpretation of the slope of disappearance of dye from the blood stream.

The rate of disappearance of dye in the blood stream is not an accurate measure of change in capillary permeability. In the experiments in this paper, local changes in permeability have been demonstrated in portions of the body large enough to affect eventually the circulating blood volume, yet the slope of dye disappearance is not significantly altered from that of normal control animals.

The experiments described in this paper confirm the gross change in capillary permeability found after a hot water burn and the concept of Drinker that lymph flow and the concentration of protein in lymph are a measure of capillary permeability. The addition of a radioactive protein to the blood stream permits sensitive detection of changes in permeability and proves them not to be false changes due to altered blood flow.

The observations on generalized permeability late in burn shock can only be considered to be preliminary. The abnormal permeability which appeared in the non-burned area late in burn shock in one experiment is without explanation. It is possible that had the dogs been more extensively burned, the remote change in permeability would have been observed more consistently. This is suggested by the experiments reported from Philadelphia (21) in which an increase in permeability in a non-burned area in a dog following an extensive burn was demonstrated by immunologic identification of foreign protein in the lymph.

The measurement of capillary permeability based upon the relation of colloid concentration in lymph and blood serum is open to erroneous interpretation when infection due to contamination in the experimental wound is present. Infectious inflammation is accompanied by altered

permeability. With the decreased blood flow of shock in peripheral tissues and the consequent anoxia, anaerobic infections flourish, and changes due to the infectious process may appear in a few hours.

SUMMARY

A study of capillary permeability has been made in dogs by injecting radioactive colloids into the blood stream and measuring their appearance in the lymph from various areas. The colloids represent tagged molecules and their concentration in the lymph is a function of capillary permeability. Radioactive brominated di-azo dyes which form a bond with plasma proteins, and brominated and iodinated radioactive plasma proteins were used.

Radioactive bromide ion is found in the leg, cervical, and thoracic lymph within 5 minutes after intravenous injection; in 20 minutes, radioactive equilibrium has been reached between these lymphs and the blood serum. This observation indicates the rapidity with which an inorganic ion traverses the capillary membrane and the interstitial space. In contrast, the rise in concentration of radioactivity in the lymph following injection of the radioactive colloids is slow and equality is not reached under control conditions.

Following a hot water burn of a leg, the concentration of radioactive colloids in the lymph from this leg rises abruptly and approaches that encountered after injection of the inorganic ion. This indicates that in a pathologic state the capillary membrane may become as permeable to colloids as it formerly was only to ions.

The ratio of concentration of radioactivity to concentration of protein (specific activity) furnishes a measure of the extent to which equilibrium has been reached in the distribution of the tagged protein in the body fluids. Under control conditions, equal specific activities are eventually reached on either side of the capillary membrane. Following a burn, a specific activity, higher in the lymph than in the blood serum, may be observed, indicating a preferential escape through the capillary wall of one fraction of the plasma protein.

Evidence of increased capillary permeability in an area remote from the burn appeared in only one experiment late in shock.

Adrenal cortical extract injected intravenously exerted no detectable effect on either local or remote changes in capillary permeability.

The authors express their gratitude to Dr. Baldwin Curtis and the Cyclotron Staff of Harvard University, and to Professor Robley D. Evans and the Cyclotron Group of The Massachusetts Institute of Technology, for providing them with radiobromine.

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TISSUE THIAMINE IN HEMORRHAGIC SHOCK[1,2]

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Govier and his coworkers (1, 2, 3) have stated that dogs previously treated with thiamine withstand hemorrhagic shock better than untreated animals. This observation has not as yet been substantiated by others, but the rise of blood pyruvate and lactate in shock suggests that tissue cocarboxylase (thiamine diphosphate), a coenzyme essential for pyruvate metabolism, may be diminished or inactivated. In order to ascertain whether thiamine metabolism is disturbed in this condition, experiments were done in which tissue thiamine was studied before and during shock in dogs.

METHOD

Thirteen dogs were studied. In 8 animals, hemorrhagic shock was induced by fractional bleeding. The remaining 5 animals were observed for 4 to 5 hours as controls; in these experiments, all procedures used in the shocked dogs were followed rigorously except that the animals were not bled.

Tissue samples were taken under local novocaine anesthesia, before and after the experimental (shock or control) period; all dogs had received 2.0 mgm. of morphine sulfate intramuscularly, approximately one hour before the beginning of the experiments. Approximately 20 to 30 grams of liver were taken for analysis. Kidney specimens were obtained by unilateral nephrectomy; the remaining kidney served for the experimental tissue. Muscle was obtained by biopsy from one of the quadriceps group of muscles. For comparison with the initial biopsy, a specimen from the contralateral group was taken at the end of the experiment. None of the animals showed a significant change from the preoperative normal blood pressure after the biopsies were taken.

The tissues were analyzed for their free (non-phosphorylated) and total (cocarboxylase plus free) thiamine content by the chemical method previously described (4, 5). In 4 animals, total thiamine was measured in the liver. In a fifth animal, both liver and muscle were thus studied. In the remaining 4 experimental animals and in the 5 control animals, liver, muscle, and kidney were analyzed for both free and total thiamine. Corrections for variations in percentage dry weight of the tissue were made in all but 2 experiments.

Fractional bleeding was used to maintain the animals' blood pressure below 70 mm. of mercury for from 1 to 6 hours; no attempt was made to ascertain the "reversibility" or "irreversibility" of shock. One animal required a small transfusion during the course of the experiment. Appended is the protocol of one typical experiment (Figure 1).

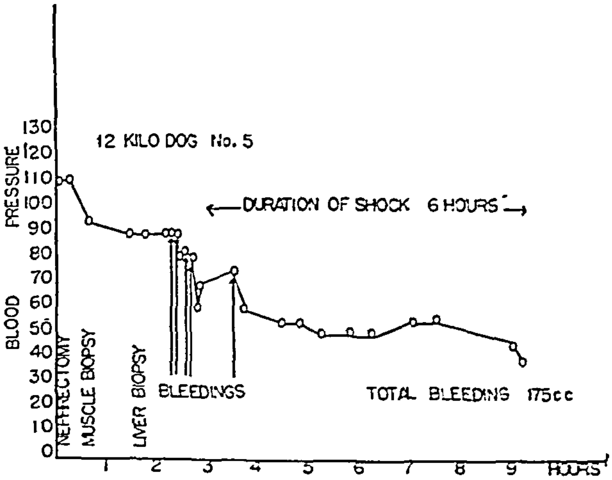

FIG. 1. PROTOCOL OF TYPICAL SHOCK EXPERIMENT

RESULTS

In 7 of 8 dogs in shock, a rise[3] occurred in the total thiamine concentration in the liver; this averaged 36 per cent (Table I). This change was due almost entirely to an increase in the cocarboxylase fraction in 3 animals so studied. The increase in liver thiamine seemed to be roughly related to the duration of shock (Figure 2). Insignificant changes, averaging plus 3 per cent, in the liver thiamine of the control animals were found (Table II).

Total thiamine concentration in the muscle showed a small decrease, and to the same extent,

[1] Reported to the Committee on Shock, O.S.R.D., Washington, D. C.

[2] This investigation has been aided by a grant from the Josiah Macy, Jr. Foundation.

[3] One animal, not included in this series, was subjected to prolonged application of a tourniquet to the legs followed by a small bleeding. In this tourniquet shocked animal, the thiamine concentration in the liver decreased 35 per cent.

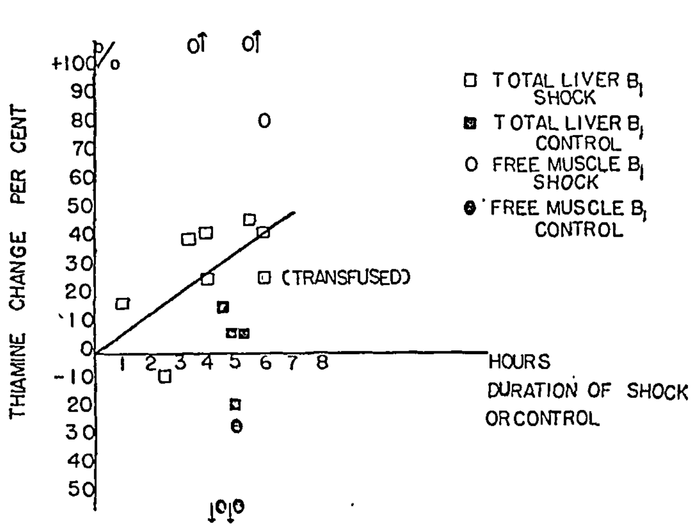

FIG. 2. LIVER AND MUSCLE THIAMINE IN HEMORRHAGIC SHOCK

in both the shock and the control groups. The free thiamine, however, rose markedly in the few shock animals so studied, in contrast to the decrease in this component in the control animals. This increase in free thiamine occurred at the expense of the cocarboxylase fraction, which in one animal (Dog 7) must have been reduced to an exceedingly low level.

Changes in kidney thiamine were extremely variable and could not be correlated with the presence or absence of shock.

DISCUSSION

Conclusions regarding the significance of the observed changes in the concentration of tissue thiamine must await further study. The changes which occur in muscle can, however, be explained on the basis of what is known about tissue cocarboxylase. The rise in the concentration of free thiamine at the expense of cocarboxylase in the muscle during shock suggests an increased *in vivo* splitting of the phosphorylated thiamine by phosphatase, with a shift to the right of the following equation:

$$\text{cocarboxylase} \rightleftharpoons \text{thiamine} + \text{phosphate}$$

TABLE I

Tissue thiamine changes in hemorrhagic shock

Dog	Time in shock	Tissue	Thiamine content						Change		
			Before shock			During shock					
			Total B_1	Cocarbox.	Free	Total B_1	Cocarbox.	Free	Total B_1	Cocarbox.	Free
	hours		*micrograms per gram*						*per cent*		
1*	1	Liver	1.29			1.54			+19.4		
2*	6 (transfusion)	Liver	0.43			0.54			+25.6		
3°	4	Liver	1.80			2.29			+27.3		
4°	4	Liver	2.13			3.02			+41.8		
		Muscle	1.50			1.04			−30.7		
5*°	6	Liver	2.69			3.84	3.29	0.55	+42.8		
		Muscle	1.73	1.45	0.28	1.68	1.16	0.51	−3.0	−19.7	+82.5
		Kidney	3.37	2.87	0.50	2.41	2.33	0.09	−28.5	−19.1	−83.0
6*°	5½	Liver	2.60	2.47	0.13	3.64	3.57	0.07	+48.5	+53.5	−43.5
		Muscle	2.20	2.13	0.072	2.00	1.60	0.40	−10.9	−25.9	+444.0
		Kidney	2.50	1.71	0.79	2.62	2.18	0.44	+3.6	+26.1	−45.0
7*	3⅙	Liver	1.71	1.44	0.269	2.45	2.11	0.342	+43.3	+46.5	+27.0
		Muscle	0.564	0.49	0.07			0.345			+392.0
		Kidney	1.79	1.58	0.214	1.65	1.275	0.375	−7.8	−19.0	+76.0
8*°	2⅓	Liver	2.70			2.50			−7.4		
		Muscle	1.70			1.68			0		
		Kidney	2.52			2.35	2.18	0.28	−6.8		

* Values corrected for any changes in percentage of dry weight of tissues.

° Animals received daily injections of 2 mgm. thiamine per kgm., for 4 or more days until day before experiment, with the exception of Dog 4 whose injections were stopped 2 days before the experiment.

TABLE II

Tissue thiamine changes in control animals—no shock

Dog	Time of experiment	Tissue	Thiamine content*						Change		
			Control			Experimental					
			Total B_1	Cocarbox.	Free	Total B_1	Cocarbox.	Free	Total B_1	Cocarbox.	Free
	hours		*micrograms per gram*						*per cent*		
1	4¼	Liver	1.52		0±			0.21			
		Muscle	2.56		0±	2.27			−11.7		
		Kidney	2.24		0±	1.81	1.77	0.04	−19.2		
2	5	Liver	1.94		0±	2.08	1.97	0.11	+7.2		
		Muscle	3.02	2.74	0.28			0.21			−25
		Kidney	2.10	1.96	0.14			0.21			+50
3	4⅚	Liver	2.01	1.80	0.208	1.65	1.60	0.044	−18.0	−11.1	−75
		Muscle			0.103	1.60		0±			
		Kidney	1.93	1.41	0.521	2.06	1.06	1.00	+6.7	−25.0	+92
4	5	Liver	4.20		0±	4.50			+7.0		
		Muscle	7.60	5.63	1.97	3.50			−54.0		
		Kidney	5.80		0±	4.40			−24.0		
5	4½	Liver	1.40			1.64	1.48	0.16	+17.0		
		Muscle	0.87								
		Kidney	2.22	1.87	0.35	2.00	1.74	0.26	−10.0	−7.0	−26

* All animals received daily injections of 2 mgm. thiamine per kgm., for 4 or more days until the day before experiment. All values corrected for any changes in percentage of dry weight of tissues.

It has been shown (6) that under anaerobic conditions, phosphatases in certain tissues, including muscle (5), destroy cocarboxylase *in vitro*. The relative tissue anaerobiosis which occurs in profound shock may thus favor splitting of cocarboxylase. These data are in agreement with the findings of Greig and Govier[4] in animals studied one-half hour after their blood pressure had fallen to 60 mm. Hg following bleeding. These authors found similar changes in animals made anoxic by breathing air containing 10 per cent oxygen in nitrogen.

Changes in the concentration of total and phosphorylated thiamine in liver are more difficult to explain. There are three mechanisms which might cause a rise in liver thiamine in hemorrhagic shock. Destruction of cocarboxylase in the muscle, with concomitant liberation of free thiamine, might result in transfer of thiamine to the liver. The absence of any decrease in the total thiamine in the muscle of shocked animals, as compared with control animals, is not inconsistent with this view, since the relatively large mass of muscle may undergo changes in the total thiamine concentration, too small to measure in that tissue by this method; the total change in all the muscles may be sufficient to cause increase in the thiamine in the liver which is so much smaller than the mass of muscle. In this connection, it should be pointed out that intravenously administered thiamine goes rapidly to the liver, where it is quickly converted into cocarboxylase (7). Other possible explanations for the increase in liver thiamine are either decreased utilization of the vitamin in this organ or decreased mobilization of thiamine from the liver to the periphery, while the liver continues to receive the vitamin from the gastrointestinal tract or from the blood, as a consequence of entry of tissue fluid into the blood stream during hemodilution consequent to hemorrhage. It must be pointed out that Greig and Govier conclude that a decrease in hepatic cocarboxylase occurs in shock. The data of their recent paper show that of the 13 experiments described, a decrease in hepatic cocarboxylase occurred in only 6, with a rise in 5 and

[4] Dr. Govier very kindly sent me the manuscript of his paper in press, Journal of Pharmacology and Experimental Therapeutics, previous to publication.

no change in 2. Their experiments are not entirely comparable with those of the present study, since their animals were under barbital anesthesia and were in shock for only 30 minutes, whereas our animals were unanesthetized and in shock for 1 to 6 hours. In addition, most of our animals were previously fortified with injected thiamine for several days before shock was induced, whereas their animals were not treated.

SUMMARY

1. The concentration of total and phosphorylated thiamine in the liver of dogs rose during prolonged hemorrhagic shock; the increase in liver thiamine seemed to be related to the duration of shock. No significant change occurred in control animals.

2. The non-phosphorylated thiamine of muscle rose markedly during hemorrhagic shock; this change occurred at the expense of the cocarboxylase.

3. The change in the free thiamine-cocarboxylase ratio in muscle is interpreted as probably consequent to *in vivo* phosphatase splitting of phosphorylated thiamine, associated with the tissue anaerobiosis occurring in shock.

Gratitude is expressed to Drs. Jacob Fine and Joseph Aub for the cooperation of their shock teams, working under O.S.R.D. contracts, and to Dr. Howard A. Frank for his assistance in the surgical procedures involved in the experiments.

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EFFECT OF SODIUM IODIDE, MAGNESIUM SULFATE, THYROXIN, AND THYROTROPIC HORMONE ON THE BLOOD MAGNESIUM PARTITION [1]

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In previous papers (1, 2), we reported the fact that, in patients with Graves' disease, there occurred a considerable increase in the percentage of bound magnesium found in the circulating blood. In normal individuals, the percentage of bound magnesium varied between 10 and 20 per cent, while in patients with Graves' disease, the percentage of bound magnesium was usually in excess of 25 per cent, and attained as high a level as 60 per cent. In patients with myxedema, the reverse was true, and in these cases, all or almost all the circulating blood magnesium was in the ionized form. Following Lugolization of the patients with hyperthyroidism, there occurred a fall in the percentage of bound magnesium, and following operation (sub-total thyroidectomy), this figure fell further to normal or even sub-normal levels. These results were subsequently confirmed by Dine and Lavietes (3), whereas Cope and Wolff (4) failed to corroborate them.

The object of this report is to present the results obtained in the investigation of certain factors which might conceivably influence the blood magnesium partition.

METHODS

Normal dogs were employed for the present investigation. Blood magnesium partition studies were carried out following the intravenous administration of sodium iodide and magnesium sulfate, and the subcutaneous injection of thyroxin and thyrotropic hormone.

The total serum magnesium was determined by the method of Briggs (5). The serum proteins were precipitated with trichloroacetic acid. It was found that little, if any, magnesium was carried down with the protein flocculum. To 10 cc. of protein-free filtrate were added 1 cc. of 20 per cent sodium acetate, 6 to 8 drops of 0.016 per cent bromcresol green, and 1 cc. of 4 per cent ammonium oxalate. The pH of the solution was adjusted to 5.0 by addition of ammonium hydroxide. The mixture was allowed to stand overnight, and the precipitated calcium oxalate then separated by centrifugation. To the decanted supernatant fluid and washings were added 1 cc. of 2 per cent potassium dihydrogen phosphate and 1 cc. of concentrated ammonia solution. After the mixture had again been allowed to stand overnight, the precipitate was separated by centrifugation and washed with a solution containing 200 cc. of 95 per cent alcohol and 50 cc. of concentrated ammonia solution per liter. The precipitated magnesium ammonium phosphate was dried and determined according to the method of Kuttner and Lichtenstein (6) by comparison of the color developed on addition of 7.5 per cent sodium molybdate and 0.2 per cent stannous chloride with that of a standard phosphate stock solution.

For the determination of diffusible magnesium, serum was ultrafiltered with the apparatus of Dine and Lavietes. The magnesium content of the ultrafiltrate was determined as described above, except that the protein precipitation with trichloroacetic acid was omitted.

RESULTS

Sodium iodide. Two dogs, numbers 313 and 420, were given 0.5 gram of sodium iodide intravenously, daily. Dog number 313 was thus treated for 31 days. The drug was then discontinued and further observations were conducted for an additional 35-day period. Total blood magnesium and magnesium partition studies were conducted at intervals of approximately 1 to 2 weeks. It will be seen from Table I that in this instance, there was practically no change in either the total or the percentage of bound magnesium. Essentially the same was true for dog number 420, similarly treated. It is interesting to note that in patients with Graves' disease, the administration of iodine results in a prompt reduction in the percentage of bound magnesium. In the normal dog, however, no such change occurs. Preliminary experiments

[1] Aided by a Grant from the Ciba Pharmaceutical Company, Summit, New Jersey.

[2] Captain, U. S. A. M. C.; Eugene Meyer, Jr., Fellow.

[3] First lieutenant, U. S. A. M. C.; Charles Klingenstein Fellow.

TABLE I

Effect of sodium iodide on total blood magnesium and percentage of bound magnesium

Dog number	Days	Total blood Mg	Bound Mg	Dog number	Days	Total blood Mg	Bound Mg
		mgm. per cent	*per cent*			*mgm. per cent*	*per cent*
313	0 (Control)	2.69	21.2	420	0 (Control)	2.64	25.3
5 cc. 10 per cent Sodium Iodide Intravenously Daily							
	12	2.77	27.8		11	1.94	20.1
	31	2.73	25.6		21	2.16	29.6
Injections Discontinued							
	55	2.73	25.6		33	2.18	25.2
	66	2.80	23.5		55	2.61	26.0

with normal humans who have been given Lugol's solution yield results similar to those obtained in normal dogs.

Magnesium sulfate. Two dogs, numbers 313 and 411, were given 3 cc. of a 25 per cent solution of magnesium sulfate intravenously, daily, for 42 days and for 75 days, respectively (Table II). The drug was then discontinued but continuing observations were carried on for an additional period of 67 days. In neither animal did the magnesium sulfate exercise an appreciable effect on the total serum magnesium or on the magnesium partition.

Thyroxin. Three dogs, numbers 313, 314, and 9, were given 4 mgm. of thyroxin subcutaneously, daily, for 27 to 30 days each (Table III). The drug was then discontinued and observations on dogs 313 and 314 were continued at intervals of 1 to 2 weeks, for an additional 32 days. Dog number 313 showed no change either in the total blood magnesium level or in the percentage of bound magnesium. Dog number 314 showed a temporary slight reduction both in the total blood magnesium and in the percentage of bound magnesium. Dog number 9 showed similar changes. The results obtained in these dogs are similar to those observed in normal obese individuals who have received thyroid extract and thyroxin for weight reduction purposes (1). Despite the administration of enough thyroid extract to produce a definite and considerable increase in the basal metabolic rate above the normal level, there occurred no increase in the percentage of bound magnesium such as was obtained in patients with true Graves' disease.

Thyrotropic hormone. Four dogs, numbers 2, 8, 308, and 314, were given 100 to 500 guinea pig units of thyrotropic hormone (Ayerst, McKenna, and Harrison) subcutaneously, daily (Table IV). Dogs numbers 2 and 8 received injections for 16 and 26 days, respectively. The experiments were then discontinued and no further observations were made. Dogs numbers 308 and 314 were injected for 24 and 34 days. The injections were then discontinued and further observations were made for 51 and 43 days.

The results obtained in 3 of the 4 dogs were essentially identical. In 3 dogs, numbers 8, 308, and 314, following the injection of thyrotropic hormone, there occurred a marked increase in the percentage of bound magnesium above the control level. This increase occurred during the first 3 weeks of injections. Following this initial definite increase, and while the injections were continued, there occurred a drop in the percentage of bound magnesium in one instance to the control level and in two instances, 308 and 314, to the point where there was no circulating bound magnesium (such as we reported to occur in myxedema in the human (1)). In these same

TABLE II

Effect of magnesium sulfate on percentage of bound magnesium

Dog number	Days	Total Mg	Bound Mg	Dog number	Days	Total Mg	Bound Mg
		mgm. per cent	*per cent*			*mgm. per cent*	*per cent*
313	0 (Control)	2.59	25.1	411	0 (Control)	2.72	21.7
3 cc. 25 per cent $MgSO_4$ Intravenously Daily							
	31	2.36	20.3		28	2.73	25.8
	42	2.38	22.3		53	2.55	22.7
					75	2.57	19.3
Injections Discontinued							
	109	2.50	32.4		96	2.06	17.4
					142	2.94	29.2

TABLE III

Effect of thyroxin on total blood magnesium and percentage of bound magnesium

Dog number	Days	Total blood Mg	Bound Mg	Dog number	Days	Total blood Mg	Bound Mg	Dog number	Days	Total blood Mg	Bound Mg
		mgm. per cent	*per cent*			*mgm. per cent*	*per cent*			*mgm. per cent*	*per cent*
313	0 (Control)	2.83	17.7	314	0 (Control)	3.05	21.9	9	0 (Control)	3.08	17.5
4 mgm. Thyroxin Subcutaneously Daily											
	18	2.88	18.1		18	2.89	13.5		20	2.67	20.2
	27	2.61	22.9		27	2.94	18.4		30	2.50	10.4
Injections Discontinued											
	36	2.86	24.1		36	2.07	17.2				
	42	2.61	27.9		42	2.41	21.2				
	59	2.69	21.2		59	2.79	22.9				

dogs, blood studies were continued after the injections were stopped, and in both instances, the percentages of bound magnesium returned to a value considerably above the original levels.

Dog number 2 received only 100 guinea pig units of thyrotropic hormone daily for 16 days. During this period of time, there occurred a decrease in the percentage of bound magnesium, not preceded by a previous increase. The experiment inadvertently was not continued beyond the injection period.

COMMENT

The object of these and experiments subsequently to be reported represents an attempt to identify those factors which may play a part in producing an elevation in the percentage of bound magnesium in Graves' disease. It is interesting that magnesium sulfate, sodium iodide, and thyroxin produce no appreciable change in normal dogs. These results, at least as far as the last two drugs are concerned, are similar to those obtained in normal human

TABLE IV

Effect of thyrotropic hormone on total blood magnesium and percentage of bound magnesium

Dog number	Days	Total blood Mg	Bound Mg	Dog number	Days	Total blood Mg	Bound Mg	Dog number	Days	Total blood Mg	Bound Mg	Dog number	Days	Total blood Mg	Bound Mg
		mgm. per cent	*per cent*			*mgm. per cent*	*per cent*			*mgm. per cent*	*per cent*			*mgm. per cent*	*per cent*
2	0 (Control)	2.51	20.0	8	0 (Control)	2.56	8.2	308	0 (Control)	2.99	12.0	314	0 (Control)	2.80	13.1
100 G. P. Units Thyrotropic Hormone Subcutaneously Daily				500 G. P. U. Thyrotropic Hormone Subcutaneously Daily											
	13	2.78	15.8		13	3.06	25.5		10	2.25	19.5		10	3.00	25.0
	16	2.62	11.8		19	2.96	12.4		20	3.05	26.9		17	2.12	17.4
					26	2.53	8.3		24	2.88	22.2		24	2.96	0.0
													34	2.48	5.2
Injections Discontinued															
									34	2.23	0.0				
									55	2.20	16.4		55	2.35	10.2
									75	2.77	28.2		77	3.05	22.3

beings. It is worth while emphasizing that the experimental results, here reported with thyroxin, are in harmony with the clinical impression that hyperthyroidism due to thyroid administration is not identical with Graves' disease.

The experimental results obtained in the dogs receiving the thyrotropic hormone are very different from those obtained with the other drugs. Following the administration of this hormone, there occurs a very considerable increase in the percentage of bound magnesium, such as we have obtained clinically only in patients with Graves' disease. This initial increase in the percentage of bound magnesium in the experimental animals is followed by a marked drop to levels seen only in myxedema in the human. This spontaneous decline in the percentage of bound magnesium, occurring during or directly after the course of the injections, would suggest that the administration of thyrotropic hormone eventually results in the development of some compensatory mechanism which vitiates the effect of the thyrotropic hormone as far as the magnesium partition is concerned. It is, perhaps, not unlikely that this may be due to the production of an anti-thyrotropic factor or hormone. This thesis is in part corroborated by results obtained in one preliminary experiment in which thyrotropic hormone was administered to a totally hypophysectomized dog. In this animal, the administration of thyrotropic hormone resulted in a persistent and sustained increase in the percentage of the bound magnesium with no tendency to fall to control or below control levels during the administration of the hormone.

SUMMARY

1. Experiments are reported on the effects of the administration of magnesium sulfate, sodium iodide, thyroxin, and thyrotropic hormone on the blood magnesium partition in normal dogs.

2. The administration of magnesium sulfate, sodium iodide, and thyroxin produced no appreciable change in the percentage of bound magnesium.

3. Injections of thyrotropic hormone produced an increase in the percentage of bound magnesium, followed by a reduction to levels considerably below the control values. After the injections of the drug were discontinued, the percentage of bound magnesium increased beyond the control levels.

4. The possible significance of these findings in relation to clinical Graves' disease is discussed.

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SULFONAMIDE-RESISTANT STAPHYLOCOCCI: CORRELATION OF *IN VITRO* SULFONAMIDE—RESISTANCE WITH SULFONAMIDE THERAPY[1]

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A feature of sulfonamide therapy for bacterial infections is that species of bacteria differ in their resistance to the antibacterial action of the compounds. It is also recognized that strains of microorganisms within the same species display variations in susceptibility. A disconcerting and confusing factor associated with chemotherapy, which appears to be assuming increasing clinical significance, is the ease and frequency with which some species of bacteria may develop *in vitro* and *in vivo* resistance to the bacteriostatic action of the sulfonamides. In the literature, the term "sulfonamide-fast" has been applied to those strains which become resistant to the antibacterial action of the compounds. This is particularly applicable to studies involving species of bacteria whose progenitors were known to be sensitive to the sulfonamides. Because the development of resistance is a relative phenomenon, and because, under proper experimental conditions, the growth of even the most resistant strains of bacteria may be inhibited by the drugs, the term "sulfonamide-resistant" is believed to be a more accurate description.

The purpose of this report is to review briefly the problem of sulfonamide-resistant bacteria in general, and to record the results of investigations with several strains of staphylococcus isolated from patients. An attempt has been made to answer the following questions: If a standard *in vitro* test is used for quantitating the inhibitory effect of the sulfonamides upon the growth of staphylococci, do strains of this species vary in their susceptibility to the antistaphylococcic action of the compounds? Is there any correlation between the isolation of sulfonamide-resistant strains of staphylococci from patients and previous sulfonamide therapy carried out in these patients? Is the development of strain resistance a permanent characteristic of the bacteria?

Several species of bacteria have been rendered resistant to the *in vitro* and *in vivo* action of the sulfonamides. Many of the investigations have been carried out with different strains of the pneumococcus (1 to 5). Sesler and Schmidt (6) observed the development of sulfonamide-resistant pneumococci, and concluded that strains vary in developing this sulfonamide-resistance; that a given strain develops resistance to the several sulfonamides at different rates; that the more susceptible a parent strain is to the action of a sulfonamide, the more difficult it is to develop resistance; and that strains which become resistant to one sulfonamide, are resistant to all the other compounds tested. There is evidence that the development of sulfonamide-resistant pneumococci is more than a temporary phenomenon (7 to 9). On the other hand, sulfonamide-resistant pneumococci are sensitive to the action of specific antipneumococcus serum. Hemolytic streptococci, particularly Lancefield group A strains, are usually quite susceptible to the action of sulfanilamide, and yet strains in this group may acquire *in vitro* and *in vivo* resistance to the drug (10, 11). While staphylococci as a species are more refractory to the bacteriostatic effect of all of the sulfonamides than are pneumococci and hemolytic streptococci, it has been demonstrated that strains of staphylococci may develop an increased resistance to the compounds (12, 13). Gram-negative species of bacteria, whose growth is usually inhibited *in vitro* by the sulfonamides, have been shown to develop sulfonamide-resistance. These include *E. coli* (14, 15), *B. abortus* (16), meningococci (17), and *Shigella paradysentariae* (strains of Flexner and Sonne) (18). Strains of gono-

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cocci, a species which is highly susceptible to the bacteriostatic action of the sulfonamides, have been made resistant to sulfanilamide and sulfapyridine (19, 20). Carpenter and his associates (21) could not develop an increased tolerance of 10 strains of gonococci to sulfathiazole over a period of 3 months. Nevertheless, this has been accomplished by Kirby (22).

If invasive strains of microorganisms develop resistance to the sulfonamides both *in vitro* and in experimental animals, the question immediately arises as to whether such a phenomenon may transpire in human subjects while they are being treated with one of the sulfonamides, and whether the development of sulfonamide-resistant strains will affect the ultimate recovery of the patient. While caution must be exercised in transposing quantitatively *in vitro* data or the results of animal protection tests with the sulfonamides to the problem of chemotherapy in human subjects, these data are often quite helpful in directing the clinical application of the drugs. Several investigators have confirmed the observation that specific types of pneumococci isolated from patients have shown an initial sensitivity to a sulfonamide, but as chemotherapy proceeded, subsequent isolation of the same type of pneumococcus revealed the development of sulfonamide-resistance (8, 23 to 28). In some instances, coincident with the detection of sulfonamide-resistant pneumococci, the patients' condition became worse or they failed to respond as anticipated. Similar observations have been made with other species of bacteria. Francis (29) encountered a group of 13 individuals on a plastic surgery ward whose lesions were infected by a group A, type 12, strain of beta hemolytic streptococcus, and the local application of sulfanilamide was without effect. *In vitro* tests showed the organisms to be resistant to sulfanilamide, although other strains of this type had been shown to be quite sensitive. It is of interest that the local application of gramicidin in one case eradicated the infection. Strains of *Brucella abortus*, sensitive to the *in vitro* action of sulfanilamide, have been made sulfanilamide-resistant by repeated exposure of the organism to the drug. Green (30) reported that 2 individuals in a laboratory became infected with these sulfanilamide-resistant strains. The patients finally recovered, although a strain isolated from the blood of one of the victims was still resistant to sulfanilamide. It was observed by Cohn and his associates (31), in gonorrheal patients who did not respond favorably to therapy with sulfathiazole, that strains of gonococci from these individuals frequently showed *in vitro* evidence of resistance to sulfathiazole. Similar observations have been recorded by Petro (32). Strains of staphylococci have been shown to develop sulfonamide-resistance in patients undergoing therapy with one of the sulfonamides (13).

As a species, the staphylococcus is generally more resistant to the sulfonamides than are several other species of pyogenic bacteria. However, several experimental and clinical studies, carried out at the University of Minnesota Hospitals, have revealed that the sulfonamides are effective antistaphylococcic agents (13, 33 to 37). As a result of these investigations, sulfathiazole has been accepted at the University Hospitals as the most effective of the available sulfonamides in the therapy of staphylococcic sepsis, but this still leaves much to be desired. While the failure of patients with staphylococcic infections to respond to therapy with sulfathiazole is dependent upon several factors, the apparent increasing incidence of patients having infections due to staphylococci which are highly resistant *in vitro* to sulfathiazole merits further inquiry.

MATERIALS AND METHODS OF STUDY

Investigations have been carried out with a total of 57 strains of staphylococci, obtained from an equal number of patients who had various types of staphylococcic infections. Isolation of the parent strains was made in brain broth or on veal agar-blood plates. The strains were then grown on slants of veal agar, having a pH of 7.8, and kept in the refrigerator until ready for transfer to a synthetic medium. Only those strains were selected for study which gave a positive coagulase test. This test provides the most simple procedure for identifying pathogenic strains of staphylococci (39). Sodium sulfathiazole was selected for testing the resistance of the microorganisms. Comparative observations with sulfanilamide, sodium sulfapyridine, sodium sulfadiazine, and sodium sulfathiazole revealed that staphylococci were inhibited in their growth to a greater degree by sodium sulfathiazole than by the other sulfonamides. Furthermore, strains that were resistant to sodium sulfathiazole were more resistant to the

aforementioned compounds. A water-clear medium of known chemical constituents, buffered to give a pH of 7.4, was employed in testing the staphylococci for their resistance to sodium sulfathiazole (40). The preparation of this medium will be described below. As far as is known, this medium has a neglible amount of sulfonamide inhibitor. A standard *in vitro* test for sulfathiazole-resistance was used throughout. Strains to be tested were grown for several generations in the synthetic medium. As will be pointed out, variable results will be obtained if the initial inoculum of bacteria is not standardized. In performing the test, 10-fold dilutions of a 24-hour bacterial suspension were made in the synthetic medium. Then 0.1 cc. of the 10^{-3} dilution was added to each of several test tubes containing the synthetic medium. The approximate number of cocci seeded to each tube was determined by making duplicate agar pour plates with 0.1 cc. of the 10^{-7} dilution. A freshly prepared, aqueous solution of sodium sulfathiazole was used. Each strain was tested against varying concentrations of the compound. This was done by starting with an initial concentration of 1 mgm. per 100 cc. and then increasing the concentration of the drug in each of a series of tubes until a maximum concentration of 360 mgm. was reached. The final total volume of each tube was 10 cc. The bacterial suspensions, with and without added sodium sulfathiazole, were incubated for 24 hours at 37° C. At the end of this period, the degree of bacterial growth was determined according to the turbidity of the contents in each tube. Sulfathiazole-resistance was quantitated by selecting the tube which showed complete inhibition of bacterial growth with the lowest concentration of sodium sulfathiazole.

In a few instances, cultures of staphylococci were isolated from patients before they had been given a sulfonamide. In the remaining cases, this was not possible because chemotherapy had been instituted before the patients were seen.

INGREDIENTS AND PREPARATION OF SYNTHETIC MEDIUM

For the preparation of 50 liters of synthetic medium, the following weighed ingredients are mixed together in a large mortar. The mixture may then be stored in a clean container in the refrigerator until ready for further use.

KH_2PO_4	225.0 grams
$MgSO_4 \cdot 7H_2O$	2.05 grams
$FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$	1.25 grams
$NaNO_3$	8.5 grams
Glucose	112.5 grams
Cystine	1.2 grams
dl methionine	1.5 grams
l tryptophane	0.51 grams
dl valine	7.5 grams
dl leucine	7.5 grams
l aspartic acid	5.0 grams
dl alanine	5.0 grams
d glutamic acid	5.0 grams
dl iso-leucine	5.0 grams
dl B phenylalanine	5.0 grams
dl lysine·2 HCl	5.0 grams
glycine	2.5 grams
l proline	2.5 grams
l hydroxy-proline	2.5 grams
l tyrosine	2.5 grams
d arginine·HCl	2.5 grams
l histidine·HCl	2.5 grams

To prepare a liter of medium, 8.2 grams of the above mixture are placed in a volumetric flask of 1 L. capacity. Twenty-six cc. of 1N NaOH are then added and 0.0337 mgm. of thiamin chloride. This quantity of thiamin chloride may be conveniently added by making up a standard solution in distilled water in which there are 10 mgm. of thiamin chloride per cc. A dilution of 1 to 296.7 is made with this standard solution, and then 1 cc. of this dilution added to the volumetric flask. Ten cc. of a M/1,000 solution of nicotinic acid are added, and then enough distilled water to bring the total volume up to 1 L. After thorough mixing in the flask, the pH of the solution is adjusted to 7.4. The solution is sterilized by passing it through a fine Berkefeld candle (size W) and collecting it in a sterile flask having a capacity of 2 L. The medium is then tested for sterility.

RESULTS

Standard in vitro *test for detecting sulfonamide-resistance*

After many *in vitro* tests for sulfonamide-resistance had been carried out, it became quite obvious that variable results would be obtained if the number of cocci inoculated into the test medium was not controlled. Furthermore, in no instance was a completely resistant strain of staphylococcus encountered. The resistance of staphylococci to the antibacterial action of the sulfonamides is relative, and the degree of resistance is directly related to the size of the inoculum. No matter how resistant a strain was, sodium sulfathiazole inhibited growth when higher concentrations of the compound were employed. The effect of the size of the inoculum upon growth inhibition by sodium sulfathiazole is shown in Table I. The strains of staphylococcus cited in Table I were considered to be sensitive to the action of sodium sulfathiazole. The results with 2 sulfonamide-resistant strains are presented in Table II. On the basis of many similar observations, the standard inoculum selected for use in all the comparative studies was 0.1 cc. of a 10^{-3} dilution of a 24-hour culture. The number of organisms in such an inoculum varied between 40,000 and 180,000 colonies.

TABLE I

Influence of size of inoculum of staphylococci upon inhibition of growth by sodium sulfathiazole
Incubation for 24 hours at 37° C.

Strain number	Size of inoculum	Concentration of sodium sulfathiazole							
		1	5	10	20	40	60	80	100
		mgm. per 100 cc.							
39	0.1 cc. 10^{-4} dilution	0	0	0	0	0	0	0	0
	0.1 cc. 10^{-3} dilution	+	+	0	0	0	0	0	0
	0.1 cc. 10^{-2} dilution	++	+	+	+	+	+	+	+
	0.1 cc. 10^{-1} dilution	++++	++++	+++	+++	+++	++	++	++
33	0.1 cc. 10^{-4} dilution	+	+	0	0	0	0	0	0
	0.1 cc. 10^{-3} dilution	++++	++	0	0	0	0	0	0
	0.1 cc. 10^{-2} dilution	++++	++++	+++	++	+	0	0	0
	0.1 cc. 10^{-1} dilution	++++	+++	+++	+++	+++	+++	+++	++
1	0.1 cc. 10^{-4} dilution	0	0	0	0	0	0	0	0
	0.1 cc. 10^{-3} dilution	0	0	0	0	0	0	0	0
	0.1 cc. 10^{-2} dilution	+	0	0	0	0	0	0	0
	0.1 cc. 10^{-1} dilution	+++	++	++	++	++	+	0	0
2	0.1 cc. 10^{-4} dilution	0	0	0	0	0	0	0	0
	0.1 cc. 10^{-3} dilution	0	0	0	0	0	0	0	0
	0.1 cc. 10^{-2} dilution	0	0	0	0	0	0	0	0
	0.1 cc. 10^{-1} dilution	++++	+++	++	++	++	++	+	+
35	0.1 cc. 10^{-4} dilution	0	0	0	0	0	0	0	0
	0.1 cc. 10^{-3} dilution	0	0	0	0	0	0	0	0
	0.1 cc. 10^{-2} dilution	0	0	0	0	0	0	0	0
	0.1 cc. 10^{-1} dilution	++++	++	++	++	++	++	+	+

0 = No growth.
++++ = Maximum growth.

TABLE II

Influence of size of inoculum of sulfonamide-resistant staphylococci upon inhibition of growth by sodium sulfathiazole
Incubation for 24 hours at 37° C.

Strain number	Size of inoculum	Concentration of sodium sulfathiazole						
		100	140	180	220	260	300	340
		mgm. per 100 cc.						
42	0.1 cc. 10^{-4} dilution	++++	++++	0	0	0	0	0
	0.1 cc. 10^{-3} dilution	++++	++++	++	0	0	0	0
	0.1 cc. 10^{-2} dilution	++++	+++	+++	++	0	0	0
	0.1 cc. 10^{-1} dilution	++++	++++	+++	+++	+++	+++	++
41	0.1 cc. 10^{-4} dilution	++++	++++	++	++	0	0	0
	0.1 cc. 10^{-3} dilution	++++	++++	++++	++	+	0	0
	0.1 cc. 10^{-2} dilution	++++	++++	++++	++++	+++	+++	++

0 = No growth.
++++ = Maximum growth.

Correlation between strains of staphylococci sensitive to sodium sulfathiazole in vitro *and the results of sulfonamide therapy*

Data for 32 strains of staphylococci, whose *in vitro* growth was inhibited by less than 1 mgm. per 100 cc. of sodium sulfathiazole, are given in Table III. These strains were considered to be the most sensitive to the antibacterial action of sodium sulfathiazole. It will be noted that 9 of 32 patients from whom the strains were isolated received one or more of the sulfonamides prior to the time when the culture of staphyloccus

TABLE III

Summary of non-resistant strains of staphylococcus

Growth inhibited by less than 1 mgm. per 100 cc. of sodium sulfathiazole

Patient and strain number	Age and sex	Type of lesion	Sulfonamide therapy prior to isolation of strain	Comments
1	78 M.	Bacteremia, Prostatitis, Thrombophlebitis	13 grams sod. sulfathiazole i.v. in 3 days.	Died. Some improvement following penicillin.
2	43 F.	Osteomyelitis, left femur	42 grams sulfathiazole, 11 days.	No improvement
3	3 M.	Impetigo, Acute hemorrhagic nephritis	None	Died
4	51 M.	Diabetes mellitus, Bacteremia, Osteo. rt. foot	34 grams sulfadiazine in 9 days. 33 grams sulfathiazole in 8 days. Sulfathiazole locally to osteo.	Recovery. Bacteremia persisted with sulfonamide therapy. Blood sterile after penicillin. Amputation rt. leg.
5	37 M.	Tbc. rt. wrist	Sulfathiazole locally for 2 months and 4 grams daily orally for 1 month. No drug for 2 months prior to obtaining culture.	No improvement from chemotherapy. Surgery, rt. wrist.
6	43 M.	Osteo. rt. femur	Large amounts sulfathiazole and sulfadiazine for 3 months.	No improvement
7	25 F.	Subacute bacterial endocarditis	None	Died. Temporary improvement from sulfapyridine.
8	23 F.	Cellulitis	None	Died. Strain produced lethal toxin.
9	16 M.	Bacteremia, Osteo. left femur	None	Recovery. No chemotherapy.
10	20 M.	Bacteremia, Osteo., Pneumonia, Empyema	Sulfanilamide, amount not known.	Died
11	39 F.	Chronic osteo. with exacerbation and bacteremia	None	Recovery. Improvement from sulfanilamide and sulfapyridine.
12	14 M.	Bacteremia, Thrombophlebitis, Meningitis	Sulfapyridine and sulfathiazole, amounts not known.	Died
13	7 M.	Chronic osteo.	None	Improvement following surgery.
14	13 F.	Chronic osteo.	None	Improvement following surgery and sulfapyridine.
15	40 M.	Chronic osteo.	None	Improvement following surgery. Questionable value of sulfonamide therapy.
16	36 M.	Chronic osteo., Perinephritic abscess, Empyema	None	Recovery following surgery.
17	84 M.	Ca bladder	None	No change
18	4 M.	Chronic osteo. left femur and humerus	Large amounts sulfapyridine and sulfathiazole, none for 6 months prior to isolating culture.	Improvement
19	7 F.	Bacteremia, Osteo., Pericarditis, Empyema	None	Recovery following penicillin therapy.
20	9 F.	Osteo., Pericarditis, Empyema	Sulfadiazine for 9 days, amount not known.	Recovery following penicillin therapy.
21	13 F.	Bacteremia, Osteo., Pneumonia	None	Recovery following penicillin therapy.
22	74 M.	Tbc. adenitis	None	Improvement
23	23 M.	Reticulo-endotheliosis, Abscess of neck	None	Died
24	26 M.	Bacteremia, Pneumonia, Osteo. of mandible, Abscess of neck	None	Recovery following surgical drainage. Also received sulfanilamide and staphylococcus antitoxin.
25	19 M.	Abscess rt. thigh	None	Recovery
26	16 mo. M.	Hydrocephalus, Meningitis	None	Recovery. Received sulfanilamide
27	14 F.	Bacteremia, Chronic osteo., Pulmonary abscesses, Meningitis	None	Died
28	32 F.	Left pyelonephritis and hydronephrosis	None	Recovery following surgery.
29	68 M.	Diabetes mellitus, Bacteremia	None	Died
30	22 M.	Actinomycosis liver and peritoneum	None	Died
31	12 M.	Bacteremia, Osteo.	None	Recovery. Received sulfanilamide and sulfapyridine.
32	26 M.	Bacteremia, Osteo.	None	Died

was isolated from the patient. In 2 of 10 patients, a sulfonamide had not been taken for several months before isolating the strain of staphylococcus (Patients 6 and 19). In most instances, treatment with the sulfonamides was instituted by physicians before the patients entered the University Hospitals. This and other circumstances did not permit us to determine the precise amount of sulfonamide that had been administered.

Since the strains of staphylococcus isolated from the patients in this series were sensitive to the *in vitro* action of sodium sulfathiazole, the next step was to analyze the effect of sulfonamide therapy upon the clinical course of these patients. Sulfonamides were administered to 10 patients after the test cultures were isolated. Patient 7 had subacute bacterial endocarditis. She was given sulfapyridine over a prolonged period of time which was associated with temporary improvement, but her clinical course ended in death. Patient 11 had a chronic osteomyelitis with an acute exacerbation and staphyloccic bacteremia. Following the administration of sulfapyridine, the blood stream became sterile and the patient improved. Chemotherapy had little effect upon the local lesion. Surgical drainage of an osteomyelitic lesion was combined with sulfapyridine in Patient 14, which was followed by improvement. This also applies to Patient 15. In both cases, it was difficult to assay the benefit of treatment with sulfapyridine. Treatment with sulfanilamide was without effect in Patient 24. Patient 26 was a small infant with staphylococcic meningitis and coincident with the use of sulfanilamide, the child recovered. Patient 31 received sulfapyridine for the treatment of acute osteomyelitis and bacteremia. Chemotherapy appeared to be definitely effective in this patient. Penicillin was given to 3 patients (Patients 1, 4, and 20), after either sulfathiazole or sulfadiazine had failed to control the infections.[2] Of the 9 patients (Patients 1, 2, 4, 5, 6, 10, 12, 18, and 20) who received one or more of the sulfonamides prior to isolation of the test culture, only one (Patient 18) appeared to benefit from such therapy.

In summary then, although a group of patients had infections due to a strain of staphylococcus which was sensitive *in vitro* to sulfathiazole, and to a less extent to some of the other sulfonamides, no consistent and outstanding therapeutic results were obtained in 13 of 32 patients who were given one of the sulfonamides.

Attention is called to the fact that several of the strains listed in Table III were isolated from patients before it had been established at the University Hospitals that sulfonamide therapy might be of definite value in selected cases of staphylococcic sepsis. Cultures of many of these strains had been maintained on veal-agar slants under oil for several months before their *in vitro* susceptibility to sodium sulfathiazole was tested. It might be postulated that some of the parent cultures of these strains may have been sulfonamide-resistant, but during the course of many subcultures, this resistance became lost. Evidence will be presented to show that the acquisition of sulfonamide-resistance by staphylococci is more or less a permanent characteristic, and that this resistance does not disappear or diminish following many subcultures.

Correlation between strains of staphylococci moderately resistant to sodium sulfathiazole in vitro *and the results of sulfonamide therapy*

A second series of 8 strains of staphylococcus were tested *in vitro* and all were found to be more resistant to sodium sulfathiazole. The results with this group are presented in Table IV. These strains required more than 1 mgm. per 100 cc. of sodium sulfathiazole and a concentration of less than 100 mgm. before growth was inhibited. In all but 3 cases (Patients 33, 37, and 40), a sulfonamide had been administered prior to isolation of the test strain. There is the possibility that Patients 37 and 40 received a sulfonamide before they were seen at the University Hospitals, but definite evidence is lacking.

In 2 of the patients (Patients 33 and 36), a culture of staphylococcus was isolated before chemotherapy, and then after a sulfonamide had been given. Patient 33 had a severe staphylococcic bacteremia associated with a thrombo-

[2] The penicillin used for experimental and clinical purposes was obtained through the Committee of Chemotherapeutic and Other Agents of the National Research Council.

TABLE IV

Summary of resistant strains of staphylococcus

Growth inhibited by less than 100 mgm. per 100 cc. sodium sulfathiazole

Patient and strain number	Age and sex	Type of lesion	Sulfonamide therapy prior to isolation of strain	Mgm. per 100 cc. of sodium sulfathiazole with complete growth inhibition	Comments
33	59 F.	Bacteremia, Thrombophlebitis	None	10	Died. Received 52 grams sulfathiazole in 8 days.
34	12 F.	Bacteremia, Cellulitis, Osteo.	174.5 grams sulfathiazole over 2-year period. Also sulfanilamide.	20	Marked improvement.
35	32 F.	Pneumonia, Empyema, Osteo. of ribs	Sulfonamide in large amounts. Quantity not known.	20	Marked improvement. Osteo. persistent after chemotherapy.
36	46 M.	Bacteremia, Retrobulbar abscess, Osteo.	195.75 grams sulfathiazole orally and parenterally. 78 grams sulfadiazine orally. Sulfathiazole locally.	5	Complete recovery.
37	64 F.	Urethritis and urethral carbuncle	None	5	Improvement after operation.
38	57 M.	Ca prostate with cystitis	21 grams sulfathiazole.	80	Died following operation.
39	32 F.	Bacteremia, Carbuncle, Osteo. of spine, tibia, fibula, and rt. femur	Large amounts of sulfathiazole for several weeks. Quantity not known.	10	Marked improvement following sulfonamide therapy. No evidence of active infection after penicillin.
40	78 M.	Ca prostate and bladder	None	20	Receiving stilbesterol.

phlebitis. Prior to treatment with sulfathiazole, the growth of a strain of staphylococcus isolated from her blood was completely inhibited by less than 1 mgm. of sodium sulfathiazole. There were 100 colonies of staphylococci per cc. in her blood when therapy with sulfathiazole was instituted. After receiving 52 grams of sulfathiazole in 8 days, she appeared moribund. The colony count of a blood culture was 473, and a strain of staphylococcus isolated from her blood required 10 mgm. of sodium sulfathiazole before growth was inhibited. The concentration of free sulfathiazole in her blood at this time was 15 mgm. per 100 cc. While care must be taken in transposing *in vitro* observations of this nature to clinical phenomena, it is not unlikely that the increase in *in vitro* resistance may have been associated in part with a fatal outcome. Similar observations were made with cultures obtained from Patient 36. This individual recovered after a very serious infection, and there is no doubt that the intensive use of sulfathiazole played a rôle in his favorable outcome. At one time, during the course of treatment, a blood culture revealed a colony count of 264 organisms per cc. In spite of the use of large amounts of sulfathiazole, the causative organism apparently developed only a minimal degree of resistance.

The results of therapy with sulfathiazole in the group of cases presented in Table IV were more satisfactory than had been obtained with sulfapyridine. This was to be anticipated, in part, following comparative *in vitro* tests with the 2 compounds when it was shown that sulfathiazole was superior to sulfapyridine in inhibiting growth of the staphylococcus.

Correlation between strains of staphylococci highly resistant to sodium sulfathiazole in vitro *and the results of sulfonamide therapy*

Table V presents a summary of 17 strains of staphylococci which are considered highly resistant to the sulfonamides. A concentration of at least 100 mgm. per 100 cc. of sodium sulfathiazole was necessary before growth was completely inhibited. All of the patients except one (Patient 54) received sulfonamide therapy prior to isolation of the test strain. In this one patient, there remains a possibility that sulfonamide treatment had been carried out before he was admitted to the hospital. An important and interesting aspect of this group of sulfonamide-resistant strains is that 14 of the 17 strains were isolated from the urine of patients having urinary tract infections. In 12 of the 14 patients with infection of the urinary tract, there

TABLE V

Summary of resistant strains of staphylococcus

Growth inhibited by 100 mgm. per 100 cc. or more of sodium sulfathiazole

Patient and strain number	Age and sex	Type of lesion	Sulfonamide therapy prior to isolation of strain	Mgm. per 100 cc. of sodium sulfathiazole with complete growth inhibition	Comments
41	13 F.	Ulcerative colitis, Chronic pyoderma	126 grams sulfathiazole orally, sulfathiazole locally.	250	Died. Only temporary improvement of skin lesions.
42	15 F.	Bacteremia, Osteo.	326 grams sulfathiazole orally over 2 years, 46 grams sulfapyridine, sulfathiazole locally.	200	Marked improvement. Residual active osteo.
43	72 M.	Bacteremia, Cellulitis, Prostatic obstruction	Large amounts sulfonamide, quantity unknown.	100	Died. No improvement.
44	74 M.	Encysted cystitis, Prostatic obstruction with cystitis	15 grams sulfadiazine. Continuous bladder irrigation with 0.8 per cent sulfanilamide solution, 5 days.	100	Improvement following operation. Resistant staph. from urine 5 times.
45	72 M.	Prostatic obstruction with cystitis	Sulfonamides at intervals for several months. 6.5 grams sulfathiazole.	200	Improvement following operation. Resistant staph. persisted in urine after operation.
46	28 F.	Rt. ureter obstructed, Pyelonephritis	Sulfonamides intermittently, amount not known.	180	Improvement following operation.
47	66 M.	Prostatic obstruction with cystitis. Suprapubic cystostomy	Sulfathiazole orally, amount not known. Sulfathiazole locally.	200	Improvement following operation.
48	65 M.	Rt. renal calculi and abscesses	38 grams sulfathiazole. 29 grams sulfadiazine.	180	Improvement following nephrectomy.
49	74 M.	Prostatic obstruction with acute pyelonephritis	51 grams sod. sulfathiazole i.v.	200	Died. Bacteremia due to gamma strept.
50	45 M.	Bilateral renal calculi with left hydronephrosis	Unknown amounts sulfonamide.	200	Improvement following surgery.
51	38 F.	Chronic pyelonephritis left with left tubo-ovarian abscess	29 grams sulfadiazine.	200	Improvement following nephrectomy.
52	4 F.	Purulent bronchiolitis with liver abscesses	21 grams sulfadiazine. 9 grams sulfamerazine.	160	Died
53	80 M.	Prostatic obstruction with cystitis	Continuous bladder irrigation with 0.8 per cent sulfanilamide solution. 5.5 grams sulfathiazole.	200	Died. Acute cardiac failure.
54	75 F.	Ca bladder, Bilateral pyelonephritis	None	160	Died. Cardiac failure after operation.
55	86 M.	Prostatic obstruction with cystitis	39.5 grams sulfathiazole.	180	Improvement following operation.
56	60 M.	Prostatic obstruction with cystitis	12 grams sulfadiazine.	200	Improvement following operation.
57	50 M.	Bilateral polycystic kidneys with uremia	17 grams sulfathiazole.	200	Died. Resistant staph. in urine 3 times.

was definite evidence of some degree of obstruction to the free flow of urine. It is well recognized that the efficiency of the sulfonamides is reduced in the treatment of urinary tract disease when a free flow of urine is interrupted. This means that in the latter group of patients, staphylococci were being constantly exposed to relatively high concentrations of one or more of the sulfonamides. It is not unlikely that under these circumstances the organisms become increasingly resistant to the compounds. Another significant feature is that this group of resistant strains, isolated from urine, were obtained over a short period of time. In 2 instances (Patients 44 and 45), sulfonamide-resistant staphylococci persisted in the urine after the obstruction had been corrected by surgical interference. Unfortunately, we were unable to obtain strains of staphylococci from any of the patients given in Table V before the administration of a sulfonamide. Several of these highly resistant strains have been investigated concerning the mechanism whereby they become resistant to the sulfonamides. This will be discussed. A definite

relationship was found to exist between the therapeutic response to a sulfonamide and the presence of sulfonamide-resistant staphylococci. In the cases of urinary tract obstruction, definite clinical improvement occurred only after the obstruction had been eliminated. The most resistant strain of staphylococcus that we have encountered was isolated from Patient 41, who had an extensive infection of the skin. She had received large amounts of sulfathiazole orally, and sulfathiazole ointment had been applied locally. There was only temporary improvement in her skin lesions and she died because of a chronic ulcerative colitis.

Summary of relationship between in vitro *susceptibility of staphylococci to sodium sulfathiazole and sulfonamide therapy prior to isolation of the strains*

A summary of this relationship is given in Table VI. Group I comprises all the strains sensitive to the action of sodium sulfathiazole. Of the 32 patients from whom these strains were obtained, only 9 had received a sulfonamide prior to isolation of the strains. There were 8 strains in Group II, and these strains were moderately resistant. Five, and possibly 7, of the 8 patients had been given a sulfonamide before the staphylococci were recovered from their lesions. There were 17 strains in Group III, all of which were highly resistant to sodium sulfathiazole. Sixteen of the 17 patients from whom these strains were isolated had had sulfonamide therapy prior to the isolation of the organisms. There is some evidence that the seventeenth patient had been given a sulfonamide. Although it cannot be concluded that the resistance of staphylococci to the inhibitory action of sodium sulfathiazole is due to previous sulfonamide therapy, it is obvious that the majority of the resistant strains were isolated from patients who had been given a sulfonamide.

TABLE VI

Summary of relationship between the in vitro *susceptibility of staphylococci for sodium sulfathiazole and sulfonamide therapy prior to isolation of the strains*

	Number of strains	Number of patients receiving sulfonamide prior to isolation of staphylococci	Comment
Group I strains—sensitive to sodium sulfathiazole.	32	9	
Group II strains—moderately resistant to sodium sulfathiazole.	8	5	Possibly 2 additional patients received sulfonamide.
Group III strains—highly resistant to sodium sulfathiazole.	17	16	Possibly seventeenth patient received sulfonamide.

Acquired sulfonamide-resistance a persistent characteristic

All of the resistant strains of staphylococci included in this report have been subcultured numerous times on veal agar slants, and in synthetic medium, and in no instance did a strain lose its ability to resist the action of the sulfonamides. This relatively permanent feature of sulfonamide-resistance is emphasized by results obtained with strains 41 and 42. As noted in Table V, these 2 strains of staphylococci were quite resistant to sodium sulfathiazole. Each of these strains was subcultured on veal agar and in synthetic medium for 75 generations. Comparative *in vitro* tests revealed that the seventy-fifth generations possessed the same degree of resistance as the parent strains. Both strains remained coagulase-positive. Strains 41 and 42 were each grown on veal agar slants, and then the cultures were covered with oil and stored in a refrigerator for 114 days. At the end of this time, the 2 strains were grown for several generations in synthetic medium and their *in vitro* resistance to sodium sulfathiazole tested. There was no diminution in the resistance of the organisms to the inhibitory effect of the sulfonamide.

DISCUSSION

The foregoing data represent the results of observations that have been made during the past 2 years. It became apparent early in the course of these studies that the mechanism whereby staphylococci developed resistance to the sulfonamides required elucidation. This effort was stimulated by the investigations of MacLeod (41) who showed that a Type I strain of sulfapyridine-resistant pneumococcus produced a substance which inhibited the action of sulfapyridine. Mirick (42) investigated this sulfonamide inhibitor and brought forth considerable evidence that this inhibitor was actually

p-aminobenzoic acid. This information suggested to us that staphylococci became resistant to the sulfonamides by means of a similar mechanism. The most resistant strains included in the present report were subjected to a group of observations with this thesis in mind. As a result, we have concluded that as far as the staphylococcus is concerned, sulfonamide-resistance is dependent at least in part, upon the elaboration of p-aminobenzoic acid by the bacterial cell. These observations will be published in detail elsewhere. This supports the conclusions of Landy and his group (43). It should be emphasized that even the so-called non-resistant strains of staphylococcus produce p-aminobenzoic acid, but to a lesser degree than the resistant strains. There is some evidence at hand which would indicate that the more resistant strains of staphylococci produce relatively large amounts of p-aminobenzoic acid, especially in the presence of the sulfonamides (44). While the mechanism whereby staphylococci resist the inhibitory action of the sulfonamides may be explained in part on the basis of the formation of p-aminobenzoic acid acting as a sulfonamide inhibitor, this mechanism does not necessarily apply to other species of bacteria. Recent evidence would indicate that another mechanism or mechanisms is responsible (9, 43).

The use of the sulfonamides in the treatment of staphylococcic infections presents many problems. Even though *in vitro* tests may show that a particular strain of staphylococcus is susceptible to the action of a sulfonamide, attempts at therapy with this sulfonamide may be unsuccessful or not too satisfactory. This is related in large part to the nature of staphylococcic sepsis. Localized lesions, serving as foci for blood stream invasion, are made up of exudate, tissue necrosis, cellular debris, and dead organisms, all of which may inhibit the action of the sulfonamide. If, in addition to these factors, the organism becomes resistant to the sulfonamide, the likelihood of controlling an infection is further reduced. Another disturbing feature in our experience with staphylococcic infections is that sulfonamide-resistant strains of staphylococci are being encountered much more frequently at the present time than 2 or 3 years ago. This may be due to several factors. One is that the sulfonamides are being administered more freely to patients with staphylococcic sepsis before they are brought to the University Hospitals for further treatment. Another possibility is that sulfonamide-resistant strains are being disseminated because of the widespread use of the sulfonamides.

Particular attention should be given to the frequency with which sulfonamide-resistant strains of staphylococci were isolated from the urine of patients with varying types of urinary tract infections. In many cases, a low-grade infection was associated with obstruction to the flow of urine. The development of sulfonamide-resistant organisms is not to be taken too lightly, particularly if operative interference is contemplated. In one patient (Patient 43), a highly resistant strain of staphylococcus was obtained from the urine. This individual had benign prostatic hypertrophy with obstruction. Sulfonamides were administered prior to surgery, and following a transurethral prostatic resection, he developed a fatal staphylococcic bacteremia. The strain isolated from his blood was also resistant to the *in vitro* action of sulfathiazole, and therapy with this compound was of no value in controlling the infection. It is possible that the same sequence of events may take place in patients with other species of bacteria in the urinary tract as brought out in the following observation. Patient 49 developed a fatal bacteremia due to an anhemolytic strain of streptococcus, following a transurethral prostatic resection. This organism was cultured from the urine and the blood, and *in vitro* tests showed it to be highly resistant to sulfathiazole. It is of interest that, in 1926, Feirer and his associates (45) called attention to the development of "drug-fast" organisms in the urine against urinary antiseptics, which were derivatives of the heavy metals. On the basis of this observation, they suggested a rotation of drugs in the treatment of chronic urinary infections.

It is becoming more and more apparent that specific agents, other than the commonly used sulfonamides, are desirable in the treatment of patients with severe staphylococcic infections. There is increasing evidence that the antibiotic agents, such as penicillin, will yield more satisfactory clinical results. We have compared the

in vitro action of sulfathiazole and penicillin against the 57 strains given in this report, the results of which work will be presented elsewhere. It is significant that p-aminobenzoic acid does not inhibit the action of penicillin against the staphylococcus. On the other hand, staphylococci may develop *in vitro* resistance to penicillin, apparently by means of a different mechanism. This feature is undergoing investigation at the present time.

SUMMARY

1. The problem of sulfonamide-resistant bacteria in general is briefly reviewed.

2. Fifty-seven strains of coagulase-positive staphylococci, isolated from an equal number of patients, were tested *in vitro* with a standard procedure against sodium sulfathiazole in a synthetic medium, containing negligible amounts of sulfonamide inhibitor. Thirty-two of the strains were considered non-resistant; 8 were moderately resistant; while 17 strains required a concentration of 100 mgm. per 100 cc. or more of sodium sulfathiazole before growth was completely inhibited.

3. The acquisition of sulfonamide-resistance by staphylococci is a persistent characteristic of the organisms.

4. Although it is apparent that sulfonamide-resistant staphylococci do not necessarily develop as a result of the administration of the sulfonamides, the evidence presented in this paper indicates that resistant strains are almost always isolated from patients who have had previous sulfonamide therapy.

5. The development of sulfonamide-resistance by staphylococci is dependent, at least in part, upon the elaboration of p-aminobenzoic acid by the bacterial cells.

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THE EFFECT OF THE INJECTION OF HISTAMINE INTO THE BRACHIAL ARTERY ON THE PERMEABILITY OF THE CAPILLARIES OF THE FOREARM AND HAND[1]

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The capillaries of the human forearm and hand are relatively impermeable to protein, and the protein concentration of normal capillary filtrate in these parts is not more than 5 per cent of the protein concentration of the plasma (1, 2). Injury makes the capillaries more permeable. In patients with peritonitis, burns, and certain forms of trauma, the capillaries in the involved areas leak protein profusely. If enough capillaries are involved, the plasma volume is decreased and shock develops. A study of the reaction of the capillaries of the forearm to the intra-arterial injection of histamine, which might be expected to make the capillaries more permeable, is, therefore, of both theoretical and practical importance.

Landis, Jonas, Angevine, and Erb (1) have described a method for determining the passage of fluid and protein through the capillary wall during venous congestion. They assumed that the loss of water from the blood would produce a proportionate increase in the packed red cell volume and in the serum protein concentration of the blood draining from the part. They made allowance for the fact that the cell volume is measured in volumes per cent of the whole blood, while the proteins are measured in the percentage of plasma. A rise in hematocrit reading without a corresponding rise in the protein concentration was interpreted as indicating that protein was escaping from the capillaries. This method was used in our laboratory to determine whether the injection of histamine into the brachial artery altered the permeability of the capillaries of the hand and forearm to protein.

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Emory University School of Medicine.

METHOD

Normal healthy males served as subjects. They rested in the recumbent position for at least 30 minutes before the experiment was begun. The hematocrit reading was measured on heparinized blood. The hemoglobin concentration was determined with a photoelectric colorimeter. The serum protein was calculated from the specific gravity by the method of Kagan (3).

RESULTS

Fifteen hundredths mgm. of histamine was injected into the right brachial artery of 2 subjects. A needle was left in place in the right antecubital vein before and for several minutes after the injection of histamine, and samples of blood were taken throughout this period.

The subjects complained of pain in the fingers, the skin of the hand and forearm became bright red and the part throbbed. In a few minutes, the finger and hand felt stiff and swollen. The reaction was confined to the extremity. There were no systemic effects. These observations were similar to those previously recorded by other workers (4). The hematocrit reading and the hemoglobin concentration of the blood draining from the forearm increased, but the protein concentration showed little change (Table I). This demonstrated that the capillaries had become permeable to protein and that plasma was passing into the tissues.

Four successful experiments were carried out, using a different technique. A blood pressure cuff was placed on the arm above the elbow. A control sample of blood was drawn without stasis. From 0.1 to 0.15 mgm. of histamine was injected intra-arterially. The pressure in the cuff was raised to the level of the diastolic blood pressure for a period of time lasting from 15 to 60 seconds. The cuff was then inflated to 200 mm. Hg. After 5 minutes, the blood in the forearm was removed by inserting a needle into

TABLE I

The effect of the injection of 0.15 mgm. of histamine into the brachial artery on the blood draining from the forearm and hand

Subject	Relation to injection of histamine	Hemoglobin	Hematocrit reading	Serum protein
		grams per 100 cc.		*grams per 100 cc.*
J. W.	Before histamine	13.9	42.6	5.8
	$1\frac{1}{4}$ minutes after histamine	14.6		6.0
	$1\frac{3}{4}$ minutes after histamine	15.0		5.8
	$2\frac{3}{4}$ minutes after histamine	14.9		5.8
	$3\frac{1}{2}$ minutes after histamine	14.6		5.8
	$4\frac{1}{4}$ minutes after histamine	14.4	43.5	5.8
	5 minutes after histamine	14.5		5.8
E. S.	Before histamine	14.2	43.8	6.2
	$1\frac{1}{2}$ minutes after histamine	15.6		6.3
	$2\frac{1}{2}$ minutes after histamine	15.4	47.0	6.2
	$5\frac{1}{4}$ minutes after histamine	14.9		6.1
	6 minutes after histamine	14.8		6.1

the antecubital vein and elevating the forearm and hand. The last portion of the blood was obtained by milking the tissues of the forearm towards the needle. By this time, from 10 to 13 minutes had elapsed since the arterial inflow was occluded. The data on the specimen of blood taken before the injection of the histamine and on the last portion of blood obtained from the forearm are given in Table II. The blood from the small vessels showed a striking rise in hematocrit reading and only a slight increase in protein concentration. Either the injection of histamine or the period of arterial occlusion or both had altered capillary permeability.

A similar type of experiment, omitting the injection of histamine and prolonging the period of arterial occlusion, was performed in 4 subjects. After the control sample was obtained, the cuff on the arm was inflated to diastolic pressure for a period of time varying from 15 to 30 seconds. The pressure was then raised to 200 mm. Hg. for from 5 to 10 minutes. The blood in the forearm was then collected by inserting a needle into the antecubital vein and elevating the arm. The last sample of blood was obtained from 9 to 15 minutes after the arterial occlusion. The data are given in Table III. The hematocrit reading and protein concentration both tended to increase and the marked discrepancy between the increase in cell volume and the increase in protein concentration, which occurred in the histamine experiments, was not present. This indicates that the majority of the changes observed in the histamine experiments resulted from the effect of histamine on the capillaries and that the accompanying ischemia had much less effect on the permeability of the capillaries.

COMMENT

Histamine produces a wheal when it is pricked into the skin. This wheal contains fluid which has a high concentration of protein (5). The experiments reported here demonstrate that histamine injected intra-arterially produces an increase in permeability in the area supplied by the artery. With the size dose employed, the effect was confined to the area supplied by the brachial artery. The same quantity of histamine injected intravenously produced no change in the hematocrit reading or protein content of the blood. The arterial blood entering the forearm was normal in composition. The venous blood leaving the forearm showed hemoconcentration because of the leakage of plasma into the tissues.

None of the patients that we have observed in shock have shown a histamine-like reaction in uninjured tissue. The erythema of the skin and the rapid swelling of an uninjured part do not

TABLE II

Effect of the intrabrachial injection of histamine on blood trapped in the forearm by a tourniquet

The venous pressure was raised before the arterial tourniquet was applied.

Subject	Relation to injection of histamine	Hemoglobin	Hematocrit reading	Serum protein
		grams per 100 cc.		*grams per 100 cc.*
J. P.	Before histamine	14.0	41.9	6.0
	10 minutes after 0.15 mgm. histamine	16.4	49.7	6.5
T. L.	Before histamine	14.7	48.5	6.0
	10 minutes after 0.10 mgm. histamine	17.0	55.4	6.4
J. W.	Before histamine	13.9	41.6	5.7
	10 minutes after 0.15 mgm. histamine	16.5	49.1	6.0
A. G.	Before histamine	17.0	48.5	6.0
	13 minutes after 0.15 mgm. histamine	21.1	60.1	6.3

TABLE III

Effect of ischemia on the blood trapped in the forearm by an arterial tourniquet

The venous pressure was raised before the arterial tourniquet was applied.

Subject		Hemoglobin	Hematocrit reading	Serum protein
		grams per 100 cc.		*grams per 100 cc.*
E. S.	Before ischemia	13.6	42.8	6.1
	After congestion and 15 minutes of ischemia	14.2	44.2	6.1
M. M.	Before ischemia	16.6	51.5	6.6
	After congestion and 9 minutes of ischemia	17.3	52.2	7.1
M. F.	Before ischemia	14.8	46.6	6.2
	After congestion and 15 minutes of ischemia	15.1	47.9	6.8
E. S.	Before ischemia	14.3	45.0	6.0
	After congestion and 15 minutes of ischemia	14.2	45.9	6.0

occur in patients with circulatory failure from burns, peritonitis, trauma, or infection. In patients dying of acute infectious diseases, the hematocrit reading and protein concentration of the arterial blood and the venous blood from the antecubital vein were compared (6). No evidence was obtained that protein was leaking into the tissues as the result of capillary injury. Patients with anaphylactic reactions have not been studied.

SUMMARY AND CONCLUSIONS

1. Histamine injected intra-arterially increases the permeability of the capillaries supplied by the artery. The rapid loss of protein from the plasma can be detected by comparing the blood draining from the part before and after the injection. The hematocrit reading and hemoglobin concentration increased markedly while the *protein concentration rose only slightly.*

2. A reaction similar to that produced by histamine is not seen in uninjured tissue in the usual types of shock.

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THE PROTEIN CONTENT OF THE EXTRACELLULAR FLUID IN NORMAL SUBJECTS AFTER VENOUS CONGESTION AND IN PATIENTS WITH CARDIAC FAILURE, ANOXEMIA, AND FEVER[1]

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No accurate measurements have ever been made of the protein content of the fluid which passes out of the vascular bed through the capillary wall. This filtrate, subject to change from resorption at the venous end of the capillary and lymph formation, makes up the extracellular fluid. In patients with massive edema, the fluid is markedly increased and pours forth when the tissues are cut. In the normal subject, this is not true because the extracellular fluid exists mainly in a jelly-like matrix. If many needles are inserted in the subcutaneous tissue of a normal subject, no detectable fluid will be found immediately in their lumina. If the needles are left in place for a period of hours, many of them will contain fluid which is yellow and has a high protein content. This fluid is almost pure plasma which has passed into the tissues because the capillaries have been injured locally by the foreign body. In other words, the capillary permeability to proteins has increased, and it is in this sense that the term will be used in this discussion. Variations in the speed of passage of various substances will not be considered. In most instances in clinical medicine, the factor of speed is relatively unimportant, for the changes leading to edema formation persist for many hours or days.

Though normal interstitial fluid cannot be obtained, it is possible to secure samples of the fluid which is formed in the legs of normal subjects as a result of elevated venous and capillary pressures. If it is assumed that this fluid contains at least as much and probably more protein than normal capillary filtrate, then determination of the protein content of this fluid will set the extreme upper limit of the protein concentration in normal capillary filtrate. Furthermore, by producing edema in patients with fever or cardiac failure, it is possible to determine whether cardiac failure or fever alters the permeability of the capillaries of the leg to protein.

Though many useful data are obtained from the analyses of interstitial fluid, it is well to recognize at the onset that this method of study has definite limitations. The composition of the extracellular fluid is the result of many variables which in active life are constantly changing. A rise in capillary pressure changes the composition of the filtrate and tends to slow resorption. Motion increases lymph flow. The amount of filtrate varies with the blood flow. The extracellular fluid is thus constantly changing in composition and varies in different parts of the body. Because of these facts, it is not possible to define the characteristics of normal capillary filtrate or extracellular fluid except within wide limits. In addition to the factors noted above, capillaries in different portions of the body are variably permeable to protein. The data reported here for the subcutaneous tissues of the leg cannot be applied without further study to other areas of the body.

METHOD

The subjects remained at bed rest throughout the procedure. No attempt was made to control the temperature of the extremities, each individual using enough cover to remain comfortably warm. The venous congestion was induced at night so that the subjects were asleep during the greater portion of the time. A blood pressure cuff, 12 cm. in width, was placed below the knee. A large reservoir with an opening in the bottom was attached to the blood pressure cuff and the entire system filled with water. The desired pressure in the cuff was obtained by raising or lowering the level of the water in the reservoir. Because the capacity of the reservoir was great in comparison to

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Emory University School of Medicine.

the volume of fluid in the cuff, movements of the subjects in bed caused little change in pressure.

At the end of 12 hours, the tourniquet was removed, and the leg elevated above the level of the heart for a few minutes. Six to eight No. 23 gauge needles were then inserted into the subcutaneous tissue and left in place for not longer than 5 minutes. The tissue pressure was increased by stretching the skin. The needles were removed and the fluid in them collected in glass capillary tubes. If the fluid was clear, it was examined under the microscope without removing it from the capillary tube. If the fluid was cloudy, or contained a large number of red blood cells, it was discarded. If there were only a few red cells, the specimen was accepted, because experience showed that the protein concentration of the fluid was not significantly affected by small numbers of cells. The total nitrogen content was determined by a modified micro-Kjeldahl method with nesslerization and colorimetric determination with a photoelectric colorimeter (1). The non-protein nitrogen of the blood serum was measured and subtracted from the total nitrogen of the edema fluid.

NORMAL SUBJECTS

Eleven acceptable samples of fluid were obtained from 7 normal subjects (Table I). The protein concentration ranged from 0.4 to 1.3 grams per cent, the average being 0.8 gram. In 4 instances, sufficient fluid was obtained to make 2 determinations, each one respresenting fluid from one or more needles. The protein concentration from the various areas differed by not more than 0.4 gram per cent.

TABLE I

Edema fluid obtained from normal subjects after application of cuff for 12 hours at 30 mm. Hg pressure

Subject	Blood	Edema fluid	
	Total protein	Obtained	Protein content
	grams per 100 cc.	*mgm.*	*grams per cent*
1	6.5	5.1	1.0
		2.9	0.8
2	6.5	4.9	0.7
3	6.9	8.6	0.7
		27.2	0.9
4	6.6	10.5	0.7
5	6.7	5.6	0.6
		5.3	0.4
6	6.4	5.4	0.9
7	6.4	2.7	1.3
		4.7	0.9
Average	6.6	7.5	0.8

The question immediately arose as to whether the local irritation from the needles caused the capillaries to become more permeable, thus raising the protein content of the edema fluid to a falsely high level. Therefore, studies were made on certain patients with comparably small amounts of edema, in whom equal difficulty was encountered in obtaining fluid. In this group, patients with cardiac failure, toxemia of pregnancy, and acute nephritis usually showed 0.1 to 0.2 gram per cent protein. It was concluded, therefore, that the method of collection did not account for the relatively high protein concentration of the edema fluid of the normal subjects.

It is impossible to say to what extent resorption of water from the tissues concentrated the protein in the edema fluid. As the edema appeared to form progressively over the 12-hour period and as the capillary pressure was quite high, it is fair to assume that resorption of water was much below normal.

Landis and his co-workers (2) studied the effect of venous congestion on the passage of fluid and protein through the human capillary wall. They calculated the relative amounts of water and protein entering the tissues of the congested part from the changes in the hematocrit reading and protein concentration of the blood draining from the part. It was concluded that, at a venous pressure of 80 mm. Hg, the capillary filtrate contained an average of 1.5 grams per cent of protein. At a venous pressure of 60 mg. Hg, very little protein loss could be detected and the capillary filtrate contained an average of 0.3 gram per cent protein.

The question as to the amount of protein present in normal capillary filtrate is not answered by the above experiments. In many patients with cardiac failure, edema forms before there is a demonstrable change in venous and capillary pressures. The protein content of cardiac edema fluid should, therefore, give an indication of the amount of protein filtering through the capillaries at more nearly normal capillary pressures. If it could be shown that the capillaries of patients with cardiac failure are no more permeable to protein than those of normal subjects, the data obtained from cardiac edema fluid could be applied to the problem of the protein content of the normal capillary

filtrate. With this in mind, patients with cardiac failure were studied.

PATIENTS WITH CARDIAC FAILURE

Edema fluid was obtained from 14 patients with cardiac failure. The protein content of fluid from the extremities varied from 0.03 to 0.6 gram per cent with an average of 0.24 gram per cent. These findings are similar to those reported in the literature (3).

In 14 patients, edema fluid was collected from 2 or more sites. The findings are recorded in Table II. In the majority of instances, the protein content of the edema fluid was approximately the same, regardless of the site. The only exception was the sacral edema fluid, which tended to be somewhat higher than that from other subcutaneous tissue. In 3 patients, edema fluid was collected on admission to the hospital, and later again when the edema had been greatly reduced by bed rest and the administration of diuretics and digitalis. These results are given in Table III. The protein content of the edema fluid showed no significant increase, as the edema decreased. This is contrary to the findings in pleural fluid where there is a marked increase in protein concentration as the fluid is absorbed (4, 5). Our findings do not confirm the increase in the protein content of subcutaneous fluid with diuresis which has been reported (5).

TABLE II

Observations on edema fluid from various sites in patients with cardiac failure

Patient	Plasma protein	Edema fluid, protein content						
		Right arm	Left arm	Sacral region	Right leg	Left leg	Right foot	Left foot
	grams per 100 cc.	*grams per cent*						
1	6.9				0.4	0.4		
2	6.6	0.2	0.3				0.2	0.2
3	6.6	0.4			0.4			
4	6.4			0.3	0.2			
5	5.6				0.3	0.2	0.4	
6	6.1			0.2			0.1	
7	7.0					0.5		0.4
8	6.5					0.4	0.2	
9	5.5	0.1	0.1		0.2			
10	6.1			0.3	0.2	0.1		
11	5.5			0.7			0.3	0.4
12	5.9			0.7			0.4	
13	6.5			0.2	0.1			
14	5.7			0.1	0.1			

TABLE III

Observations on edema fluid of patients with cardiac failure before and during compensation

Patient	Date	Edema fluid, protein content	Remarks
		grams per cent	
1	September 29, 1941	0.3 to 0.4	Much edema
	October 1, 1941	0.4 to 0.5	Slight edema
	April 22, 1942	0.2 to 0.3	Return of edema
2	October 1, 1941	0.2 to 0.4	Edema
	October 7, 1941	0.3 to 0.4	Slight edema
3	December 30, 1941	0.3 to 0.4	Much edema
	January 3, 1942	0.2	Less edema
	January 9, 1942	0.3	Barely visible edema

The data recorded above indicate that, in general, the protein content of cardiac edema fluid is considerably lower than that obtained in normal subjects after venous congestion. The plasma protein levels were not significantly different in the two groups. It is probable, therefore, that the capillary filtrate in normal subjects is much lower at the usual capillary pressure than at the higher pressures used by us in producing edema fluid in normal subjects. Various authors have assumed that the capillaries in patients with heart failure are abnormally permeable to protein. If this were true, the capillary filtrate in normal subjects would contain less protein than that obtained from patients with congestive failure. To determine whether the capillaries of the leg in patients with heart failure were more permeable to protein than those of the normal subjects, the following experiments were performed.

The protein content of the edema fluid already present in the lower extremities was first determined. Digitalis and diuretics were given until the edema disappeared. The blood pressure cuff was then applied below the knee and inflated with a pressure of 30 mm. Hg, for 12 hours. At the end of this time, edema fluid was collected and the protein content determined. Three such experiments were performed. The protein content of the edema fluid before diuresis was 0.3, 0.1, and 0.4 gram per cent. The protein content of the edema fluid after 12 hours' congestion was 0.4, 0.7, and 0.9 gram per cent, respectively. These data indicate that the

capillaries of the leg in patients with cardiac failure are no more permeable to protein than the vessels of normal subjects.

The above experiments suggest that the protein content of the capillary filtrate of patients with congestive heart failure does not exceed that of the normal subject. The protein content of normal capillary filtrate is probably considerably lower than that of cardiac edema fluid, for even in heart failure some resorption of fluid by the venous end of the capillary may take place. The finding that the edema fluid formed by venous congestion in normal subjects contained an average of 0.8 gram per cent of protein suggests that the protein leakage is increased as the capillary pressure rises. It is not possible to say whether the variations occurring from subject to subject in the protein content of the edema fluid result from differences in capillary permeability, in absorption of the fluid by the venous capillaries, or in lymph flow.

It has been noted previously that venous congestion produces edema more readily in patients with cardiac failure than in normal subjects (6). This observation has no bearing on capillary permeability. The capillaries are normally permeable to salt and water so that edema may form in the presence of normal capillaries. The more rapid accumulation of salt and water in the tissues of patients with congestive failure does not indicate that the capillaries are abnormally permeable.

PATIENTS WITH ANOXEMIA

Two patients with marked emphysema and arterial unsaturation were studied. The arterial blood was only 50 per cent and 60 per cent saturated with oxygen. The patients were cyanotic, confused, and uncooperative. The mental state improved with the administration of oxygen. They were orthopneic and developed pitting edema of the ankles. The protein content of the edema fluid was 0.2 and 0.1 gram per cent, respectively. The marked anoxemia had not increased the permeability of the capillaries of the lower extremities. It is well known that patients with marked arterial oxygen unsaturation from congenital heart disease do not develop edema unless their circulation fails. It is concluded that the oxygen unsaturation arterial blood must reach a level below required to nourish the brain before cap permeability in the leg is affected to such a d that it is clinically significant. Death d injury to the nerve cells occurs before the laries have been damaged sufficiently to protein. Tissue anoxia can result from marked slowing of the blood flow, even in presence of normal arterial oxygen satura Does this stagnant anoxia cause capi damage and loss of plasma from the b. stream? This has been studied in the s circulation caused by marked cardiac failure These patients showed as profound an im ment in the circulation as that seen in pa with advanced shock. Measurements of plasma volume, the hematocrit reading, protein concentration of the plasma, and protein content of edema fluid indicated the majority of the capillaries of the body not abnormally permeable to protein. Lc changes in capillary permeability in one org could be overlooked by this method of stud In generalized stagnant anoxia, the cells of brain and other organs become functionally i competent before the capillaries have becc abnormally permeable.

This does not mean that *local* anoxia canno produce injury to a part with resulting incr in capillary permeability. Any form of injur to the capillaries, if severe enough, will increa their permeability to protein. In practice, marked local anoxia is associated with a deficient supply of the other constituents of the blood, and it is difficult to tell what portion of the cell damage is the result of anoxia alone.

PATIENTS WITH FEVER AND INFECTION

The effect of fever and infection on capillary permeability in the leg was studied. Edema in the leg was produced by a 12-hour period of venous congestion, with a tourniquet inflated to a pressure of 30 mm. Hg. In 5 patients, the protein content of this fluid ranged from 0.3 to 0.9 gram per cent, the average being 0.5 gram per cent (Table IV). This indicated that the capillaries in the leg were not made more permeable to protein as a result of fever and infection.

TABLE IV

Edema fluid obtained from patients with fever after application of cuff for 12 hours at 30 mm. Hg pressure

Patient	Plasma, total protein	Edema, total protein
	grams per 100 cc.	*grams per cent*
1	6.7	0.3
2	5.1	0.3 to 0.5
3	5.2	0.3
4	6.5	0.6 to 0.7
5	6.2	0.9
Average	5.9	0.5

The above conclusion is supported by our previous observations on the plasma volume changes in patients dying of acute infectious diseases (8). When the patients received an adequate fluid intake, there was no decrease in plasma volume and no fall in the plasma protein concentration. This is interpreted as showing that the infection had produced no generalized increase in capillary permeability. It does not mean that the capillaries are not abnormally permeable in local areas, for example in the lung in pneumonia. It does mean that the body is able to add new protein to the blood stream as rapidly as it is lost so that the circulation as a whole is not affected.

SUMMARY AND CONCLUSIONS

1. The filtrate from the capillaries of the skin and subcutaneous tissues normally contains some protein. On the average, it does not contain more than 0.24 gram per cent of protein. It probably contains much less.

2. Elevation of the venous pressure in the leg to a level equal to 30 mm. Hg produces edema which contains from 0.4 to 1.3 grams per cent of protein, with an average of 0.8 gram per cent.

3. Cardiac failure does not make the capillaries of the leg more permeable to protein.

4. Generalized anoxemia, sufficient to cause impaired cerebral function, does not cause increased permeability of capillaries in the leg. Although local ischemia produces capillary damage and leakage of protein, generalized stagnant anoxia of a degree compatible with life does not make the capillaries of the leg more permeable to protein.

5. Fever and acute infectious disease cause no abnormal increase in permeability to protein in the capillaries of the leg.

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THE LEVEL OF VITAMIN A AND CAROTENE IN THE PLASMA OF RHEUMATIC SUBJECTS

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It is recognized that rheumatic fever occurs more frequently in individuals from low income groups than in those from economic groups with greater income. In a recent study (1), the diets of 50 rheumatic children from families of low income were analyzed and compared with those of 50 non-rheumatic children from families on high income. Striking differences were found. The diets of the rheumatic children contained less than required amounts of various food components, with most marked inadequacies of protein, iron, and vitamin A. The occurrence of inadequate intake of vitamin A was much more frequent in the rheumatic than in the non-rheumatic children, only 2 of the former and 36 of the latter having diets deficient in this component. There was a statistically significant association between susceptibility to rheumatic fever and inadequate intake of vitamin A. In the half of the rheumatic group considered most susceptible to rheumatic fever, only one child received vitamin A in required amounts. An analysis of the diet in terms of units of vitamin A obtained from animal and plant sources revealed the following facts: (*a*) children most susceptible to rheumatic fever received an average of 2280 I.U.[4] from animal and 2340 I.U. from vegetable sources, daily; (*b*) corresponding values for children less susceptible to rheumatic fever were 4840 I.U. and 4900 I.U., respectively; (*c*) non-rheumatic children from families of high income groups received 10,580 I.U. from animal and 4960 I.U. from plant sources. It was not demonstrated, however, that a deficiency in this single component of the diet bore a causal relationship to rheumatic attacks (1). The purpose of the present paper is to record the results of further study of the relation of vitamin A to the rheumatic state.

METHODS

Concentrations of vitamin A and carotene in plasma were determined by Kimble's modification (2) of the method of Dann and Evelyn; the Evelyn photoelectric colorimeter was used in these determinations. To 5 cc. of blood plasma in a 25 cc. centrifuge tube with a ground glass stopper, an equal volume of 95 per cent ethyl alcohol was added to precipitate the proteins. Extraction of vitamin A and carotene was accomplished by the addition of 12 cc. of petroleum ether. The glass stopper was inserted and sealed with a drop of mineral oil. The tube was inverted repeatedly for a period of 10 minutes and centrifuged at 1000 R.P.M. Ten cc. of the petroleum ether layer were removed and the carotene was determined directly in the photoelectric colorimeter by means of color filter No. 440 and a petroleum ether blank. The concentration was ascertained by reference of the galvanometer reading to a standard curve, prepared from known concentrations of crystalline carotene, containing 90 per cent beta and 10 per cent alpha carotene. After reading the value of the carotene color, the petroleum ether extract was evaporated to dryness in a stream of warm air in a water bath at 45° C. The residue was dissolved in 1 cc. of chloroform and 9 cc. of 25 per cent antimony trichloride in chloroform were added. Since the blue color of the test fades rapidly, it was developed with the tube in the colorimeter block. The maximum value of the color was read with filter No. 620 and against a blank of 25 per cent antimony trichloride in chloroform. Values of vitamin A were obtained from a standard curve prepared by use of a distillate of the vitamin in natural ester form. $E^{1\ \text{per cent}}_{1\ \text{cm.}}\ 328$ of this preparation had been determined to be equal to 114.8 and the conversion value of $E^{1\ \text{per cent}}_{1\ \text{cm.}}\ 328 = 1$ was 1850 I.U. vitamin A. The blue color produced by the plasma carotene was corrected for by a factor relating

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[4] International units.

the intensity of the color produced by antimony trichloride to a known concentration of crystalline carotene. The method gave reproducible results and only small percentage errors on duplicate determinations. The blood samples on which the determinations were made were not fasting samples but were taken in the morning 2 to 4 hours following the morning meal. Kimble (2) has shown that ordinary meals cause no measurable increase in the concentration of vitamin A or carotene of the plasma within 2 to 6 hours of ingestion. May, Blackfan, McCreary, and Allen (3) found very little diurnal variation in the blood level of vitamin A or carotene of subjects on usual diets.

Erythrocyte sedimentation rates were determined frequently on all of the patients with acute rheumatic fever. The method of Westergren (4) was used.

RESULTS

Estimation of vitamin A in diets

The necessity of caution in relying upon dietary histories in a survey of this kind was appreciated (5). There are probably discrepancies between food actually consumed and the amount recorded by the subject. It is believed, however, that errors occurring in this study appear throughout in a truly random manner. The objective of obtaining a week's record of all food ingested by each subject was explained to a responsible member of every family cooperating in the study. Families were requested not to alter their diets in any way. Visits were made by a trained field worker during meal hours and, when cooperation seemed satisfactory, forms were left to be filled in after each meal. These forms with simple instructions for recording the kinds and amounts of food consumed, were prepared by a dietitian who assisted in the dietary analysis. From the data collected, the vitamin A consumed was calculated according to published compilations (6, 7). Vitamin preparations, used in reinforcing the diets, were included in the calculations. The excess or deficiency for each individual was computed by comparison with the requirements for age and sex of the child, as proposed by the National Research Council (8).

The relation of levels of vitamin A and carotene in the plasma to vitamin A ingested

Levels of vitamin A and carotene in the plasma were determined on 4 groups of subjects whose intakes of vitamin A had been calculated. The first of these groups included 12 normal children, of families in the high income class. The average daily intake was 109 per cent above the recommended amount for age and sex. The other 3 groups were composed of rheumatic children, no longer evidencing signs of disease activity, but on diets containing varying quantities of vitamin A. In the second group were 24 children who received more than required amounts of vitamin A, their diets averaging 23 per cent above the calculated requirements. Forty-six subjects made up the third group. They received vitamin A in adequate amounts,

TABLE I

The relation between diet and the plasma level of vitamin A and carotene

Subjects	Number of subjects	Vitamin A in plasma, high level	Vitamin A in plasma, low level	Vitamin A in plasma, median level	Carotene in plasma, high level	Carotene in plasma, low level	Carotene in plasma, median level	Intake of vitamin A
		I.U. per 100 cc.			*mgm. per cent*			
1. Diets high in vitamin A Normal children	12	194	116	139	0.258	0.060	0.136	+ 109 per cent of calculated requirements
2. Diets high in vitamin A Rheumatic children	24	204	84	132	0.319	0.088	0.154	+ 23 per cent of calculated requirements
3. Diets adequate in vitamin A Rheumatic children	46	180	65	108	0.223	0.043	0.112	+ 5 per cent of calculated requirements
4. Diets low in vitamin A Rheumatic children	25	168	41	93	0.132	0.033	0.084	− 44 per cent of calculated requirements

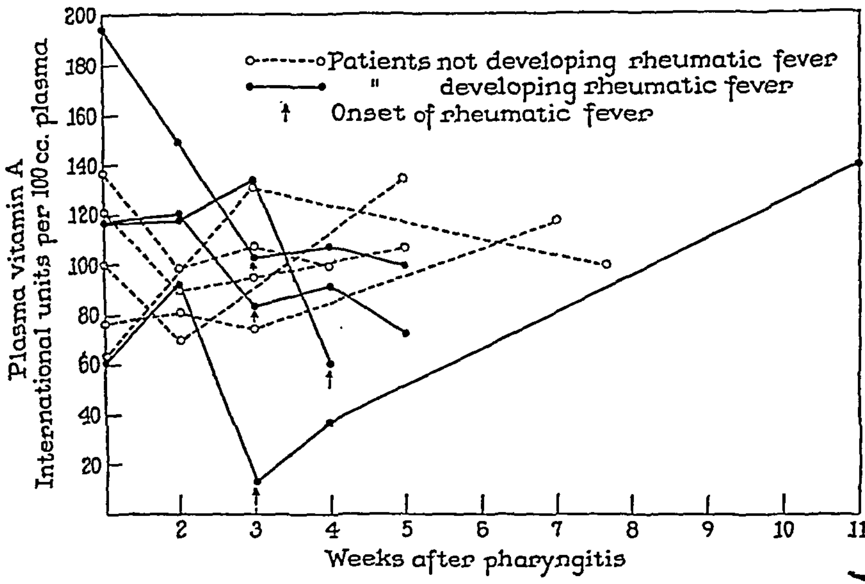

FIG. 1. LEVELS OF VITAMIN A IN PLASMA FOLLOWING ACUTE PHARYNGITIS AND AT THE ONSET OF ACUTE RHEUMATIC FEVER

with an average intake 5 per cent in excess of requirements. The fourth group included 25 children on low levels of intake, the average for the group being 44 per cent less than the optimum intake. The relation between intake and concentration of the vitamin and carotene in the plasma of these 4 groups is shown in Table I.

Although the range of concentration in each group was wide, the data in Table I show that there is a relationship between intake of vitamin A and its level in the plasma. The subjects of the first 3 groups, whose diets contained more than the required amounts of vitamin A, had plasma concentrations which, with but few exceptions, fell in the range of 100 to 200 I.U. per 100 cc. plasma. When the dietary intake exceeded the required amount, there was not a proportionate increase of vitamin A in the plasma. Most of the subjects in Group 4, who were on poor diets, containing only 56 per cent of the recommended daily intake, had levels below 100 I.U. per 100 cc. plasma.

Concentrations of carotene in the plasma varied widely in each of the 4 groups of subjects. However, the range of concentration was smallest in Group 4 and the median level was 0.084 mgm. per cent for this group, compared with 0.136 mgm. per cent, 0.154 mgm. per cent, and 0.112 mgm. per cent, median levels for Groups 1, 2' and 3, respectively. Therefore, in the groups of subjects studied, the concentration of vitamin A and of carotene in the plasma was lowest in the group whose diets contained the smallest quantities of vitamin A.

The relation of levels of vitamin A in the plasma to the development of rheumatic fever

Repeated determinations of vitamin A in the plasma were made on 9 rheumatic children during periods of pharyngitis caused by Group A *beta* hemolytic streptococci and for several weeks following recovery. Five of these children failed to develop a recrudescence of rheumatic fever while 4 manifested signs of acute rheumatism about 3 weeks following the onset of the infection of the respiratory tract. The curves of concentrations of vitamin A in the plasmas of these 9 patients are presented in Figure 1.

In Figure 1, it is seen that patients with acute pharyngitis had low levels of plasma vitamin A during the acute infection, and that with recovery, these levels increased. The onset of rheumatic activity in 4 subjects was accompanied in each case by a fall in the concentration of vitamin A in the plasma to a level lower than that which was found during acute pharyngitis.

The relation of levels of vitamin A and carotene in the plasma to rheumatic activity

Frequent determinations were made of the concentration of vitamin A and carotene in the plasma of 57 children with rheumatic activity. The degree of intensity of the disease was classified as being severe, mild, subsiding, or convalescent, according to commonly accepted clinical signs and laboratory findings. The results are shown in Table II.

It is seen from the results in Table II that values for vitamin A in the plasma were lowest in those patients exhibiting greatest rheumatic activity. During less severe attacks, levels of vitamin A were also low. Low levels in the plasma were not always associated with pyrexia; many of the patients with severe attacks were afebrile when blood samples were obtained. Although there was a wide range of concentration during the periods of subsiding rheumatism and convalescence, the concentration tended to return gradually to normal. Throughout the course of the disease, the changes in level of vitamin A in the plasma were inverse to the changes in the rate of sedimentation of erythrocytes, plasma concentration of vitamin A being low when sedimentation rates were high and increasing with decreasing sedimentation rates. Patients included in the group with most severe attacks of rheumatic fever had sedimentation rates greater than 40 mm. per hour. The rates in the patients with mild attacks fell between 20 and 40 mm. per hour, while in the patients in whom the disease was subsiding, they did not exceed 20 mm. per hour. In the convalescent patients, the sedimentation rate was less than 15 mm. per hour. There was no correlation between concentration of carotene in the plasma and the activity of the rheumatic process, median levels being very nearly the same in severe, mild, subsiding, and convalescent phases of the disease.

Excretion of vitamin A in the urine and changes in levels of vitamin A in the plasma following large single doses of carotene and of vitamin A in patients with rheumatic fever

The results of this study have shown that there is a relationship between rheumatic activity and low concentration of vitamin A in the plasma and that the blood level of this vitamin falls abruptly with the onset of acute rheumatic fever. It was assumed, therefore, that in acute rheumatic fever there is a deviation from normal in the metabolism of vitamin A. To investigate this change, further studies were made of vitamin A excreted in the urine and also of changes in levels in the plasma following large single doses of carotene and vitamin A. The patients with acute rheumatic fever were receiving therapeutic doses of salicylates when these tests were done. Both the normal subjects and the rheumatic patients were permitted their usual meals on the days that test doses of carotene and vitamin A were given. Only small portions of butter, cream, and other fatty foods were allowed on these days, however.

Vitamin A is not found in the urine of normal subjects (9) but has been found in the urine of women during pregnancy (10, 11) and of patients with chronic nephritis, nephrosis, cirrhosis of the liver, cholecystitis, pneumonia before crisis, tuberculosis, and cancer (9 to 12). Urine speci-

TABLE II

The relation between rheumatic activity and the plasma levels of vitamin A and carotene

Intensity of rheumatic activity	Number of patients	Vitamin A in plasma, high level	Vitamin A in plasma, low level	Vitamin A in plasma, median level	Carotene in plasma, high level	Carotene in plasma, low level	Carotene in plasma, median level
		I.U. per 100 cc.			*mgm. per cent*		
Severe	16	70	0	46	0.259	0.036	0.098
Mild	11	103	52	75	0.154	0.067	0.092
Subsiding	18	156	61	88	0.221	0.017	0.108
Convalescent	12	162	33	93	0.120	0.092	0.101

mens from 6 children with rheumatic fever were collected for periods of 24 hours and determinations made of vitamin A according to a modification of the method described for the determination of the vitamin in the blood (9). Each of these specimens contained traces of substances which gave a blue color with antimony trichloride, but in no instance was there more than that given by 20 I.U. of vitamin A excreted in a 24-hour period.

In order to study the changes brought about in the concentration of vitamin A in the plasma by large single doses of carotene and vitamin A, test doses of these substances were given to rheumatic and to normal children. Carotene in oil was given orally to 4 patients with acute rheumatism and to 4 normal children; a test dose, representing 200,000 I.U. of vitamin A activity, was administered to each subject. Levels of carotene and of vitamin A in the plasma were determined immediately prior to the ingestion of the test dose and at periods of 2, 4, 6, and 24 hours afterward. Although concentrations of plasma carotene were slightly increased after 24 hours, there were no significant changes in levels of vitamin A either in the rheumatic patients or in the normal children. Therefore, it was not possible to study the rate of conversion of carotene to vitamin A in these patients by this means.

To 7 children with rheumatic fever and to 6 normal children, 200,000 I.U. of vitamin A, as a distillate in the form of the natural ester, were given by mouth. Levels of vitamin A in the plasma were determined prior to the administration of the test dose and at intervals during the following 24-hour period. The results are presented in Figure 2.

Highest concentrations of vitamin A were reached in from 5 to 8 hours after the administration of the test dose of the vitamin (Figure 2). In normal children, the peak concentrations fell between 1340 and 2400 I.U. per 100 cc. plasma. In rheumatic fever patients, highest levels were from 274 to 1150 I.U. per 100 cc. plasma. This would seem to indicate a decreased rate of absorption of vitamin A from the intestinal tract or an increased rate of removal from the plasma in rheumatic fever.

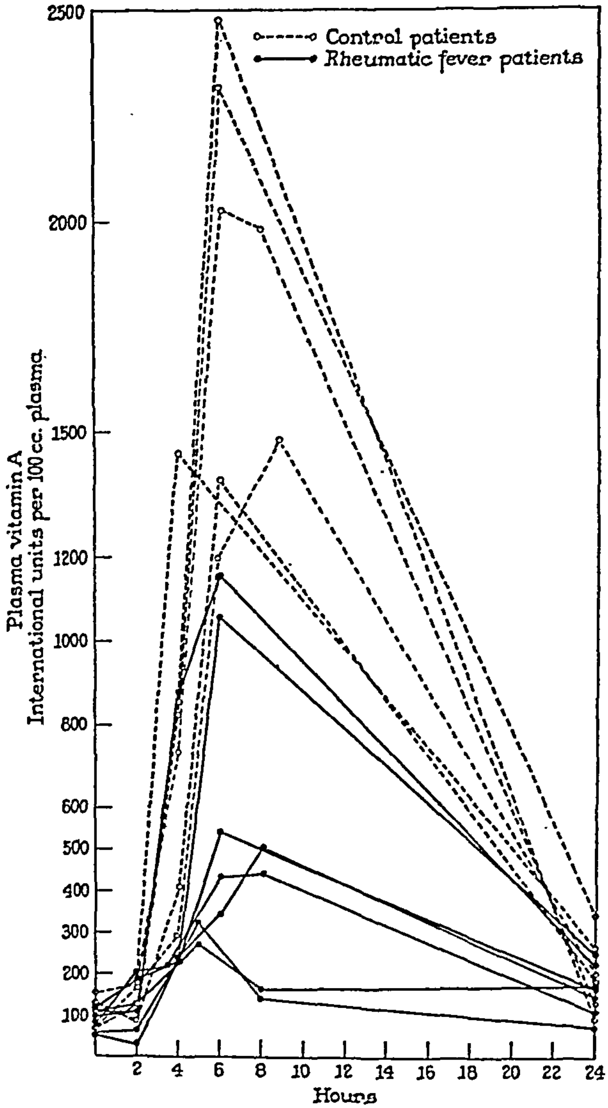

FIG. 2. PLASMA LEVELS OF VITAMIN A FOLLOWING ADMINISTRATION OF 200,000 I.U. VITAMIN A BY MOUTH

DISCUSSION

In an earlier report (1), dietary analyses had shown that there was a statistically significant association between susceptibility to rheumatic fever and inadequate intake of vitamin A in the diet. The incidence of rheumatic fever is greatest in individuals of the low income groups. The lack of information concerning dietary requirements and the relatively high cost of foods rich in vitamin A probably account for the deficiencies in the diets of persons in these economic groups. In the studies reported in this paper, it was found that children who were susceptible

to rheumatic fever and who were on diets containing adequate amounts of vitamin A had concentrations of vitamin A and carotene in the plasma comparable to those of normal children on adequate intake. The rheumatic children whose diets did not provide vitamin A in required amounts had low concentrations of the vitamin and of carotene in the blood plasma. May, Blackfan, McCreary, and Allen (3) and Josephs, Baber, and Conn (13) previously reported low levels in normal children on inadequate diets.

During pharyngitis caused by Group A *beta* hemolytic streptococci, the concentration of vitamin A in the plasma of rheumatic subjects was low. With subsidence of the pharyngitis, the concentration increased but dropped again to low levels if acute rheumatic fever ensued. The lowest levels of vitamin A were found in patients with the most severe attacks of rheumatic fever. There was a gradual increase in the vitamin A level in the plasma during recovery from the rheumatic attack. Blood carotene concentrations were not significantly changed by disease activity. The mechanism which brings about these changes in concentration of vitamin A in the plasma was not revealed. However, it was found that test doses of vitamin A, given by mouth, produced less increase in the concentration of vitamin A in the plasma of children with acute rheumatic fever than in normal subjects. There was no loss of unusual amounts of the vitamin in the urine.

It is known that in pneumonia and in other acute infections (3, 14 to 17), both the level in the blood and the concentration of vitamin A stored in the liver are decreased. Ellison and Moore (17) determined the reserves of vitamin A stored in the liver in 200 children dying by accident or from various diseases and found the lowest reserves in children dying of valvular diseases of the heart. The requirements for vitamin A are increased in thyrotoxicosis (18) and in pregnancy (19). Fever, produced artificially (20), is accompanied by a decrease in the concentration of the vitamin in the plasma. It would seem, therefore, that conditions resulting in increased demands on general body metabolism are accompanied by a decrease in plasma level of vitamin A. Whether vitamin A is destroyed in some abnormal manner in these conditions, or whether it is utilized in normal metabolic processes but with increased velocity, can be determined only when more knowledge is available concerning the mode of action of vitamin A.

SUMMARY

1. The level of vitamin A and carotene in the plasma is related to the intake of vitamin A in the diet of rheumatic subjects.

2. *Irrespective of the concentration prior to* the onset of disease activity, there is a fall in the level of vitamin A in the plasma with the development of acute rheumatic fever. The concentration of carotene in the plasma is not significantly changed during rheumatic attacks.

3. The degree of decrease of vitamin A in plasma varies directly with the intensity of the rheumatic attack. In severe attacks, concentrations in the plasma varied between 0 and 70 I.U. of vitamin A per 100 cc. plasma.

4. Patients with rheumatic fever show delayed or decreased absorption of vitamin A or metabolize it in an abnormal manner.

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SUBCLINICAL VITAMIN DEFICIENCY.[1] IV. PLASMA THIAMIN

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(Received for publication July 15, 1943)

INTRODUCTION

The possibilities for assaying directly by laboratory measurements the nutritional status of an individual with respect to his thiamin nutrition may be divided into several categories: determinations of intracellular thiamin, determinations of extracellular thiamin, and determinations of thiamin excretion. Determinations of thiamin excretion have been used extensively (1 to 5), determinations of extracellular and intracellular thiamin much less frequently (6 to 11). The following investigation represents an effort to explore the possibility of using measurements of extracellular thiamin, in this case plasma thiamin, as an index of thiamin nutrition.

Because the major portion of blood thiamin is bound in the cellular constituents (12), and in view of the recognized limitations of methods previously utilized (7, 9), the micro-yeast fermentation method was used to determine the plasma thiamin (10). In using this method, it is necessary to distinguish between "true" thiamin and non-thiamin yeast-stimulating substances (12). This is particularly true where there is a wide range of thiamin values of low magnitude and where the non-thiamin moiety represents as much as 65 per cent of the total activity measured.

The necessity for determining the blank was recognized early in this investigation. Preliminary trials with the sulfite technic (13) proved unsatisfactory. Attempts were made to destroy the plasma thiamin using a purified carp "anti-thiamin enzyme," prepared from dried carp intestines (14). This method failed because of the introduction of new yeast-stimulating substances in the enzyme preparations used.[2] It was finally necessary to revert to the sulfite procedure, herein described, in spite of its unsatisfactory characteristics. The justification for this course lies in the fact that while an error of 20 per cent may result, the clinical variations sought are two or three times as great.

METHOD

Care is taken to avoid hemolysis of the samples. Blood is drawn into tubes containing heparin, and immediately centrifuged at high speed. Gorham *et al.* (12, p. 163), in a study of leukocyte thiamin, have shown that although there is fragmentation of cells, no liberation of thiamin into plasma takes place. Significant hemolysis would yield falsely high values. Two or 3 cc. of plasma from the

TABLE I

Cubic millimeters of carbon dioxide produced in one hour by yeast suspension in thiamin standard samples and in plasma samples

Sample	Manometer readings: Initial	Manometer readings: Final	Corrected Δ in pressure	Constant	CO_2
	mm.	*mm.*	*mm.*		*c.mm.*
Blank	151	191	42	1.57	66
Blank	151	195.5	46.5	1.39	65
2 millimicrograms thiamin	151	212	63	1.47	93
2 millimicrograms thiamin	150	212	64	1.39	89
3 millimicrograms thiamin	151	220	71	1.43	102
3 millimicrograms thiamin	150	224	76	1.33	101
4 millimicrograms thiamin	150	222	74	1.54	114
4 millimicrograms thiamin	150	227	79	1.42	112
5 millimicrograms thiamin	151	229	80	1.53	122
Diluted plasma (0.2 cc. plasma)	151	215	66	1.55	102
Diluted plasma (0.2 cc. plasma)	151	221	72	1.45	104
Diluted plasma (0.2 cc. plasma)	151	219	70	1.49	104
Barometer	150	148			

[1] Aided by a grant from the Williams and Waterman Fund, Research Corporation, New York City.

[2] These carp preparations destroyed all thiamin in 10 and 25 microgram samples, incubated one half hour, at 45° C. at pH 7.4, as tested by the thiochrome fluorescence method. An aliquot of the same solution, suitably diluted, always gave some yeast stimulation above the control values.

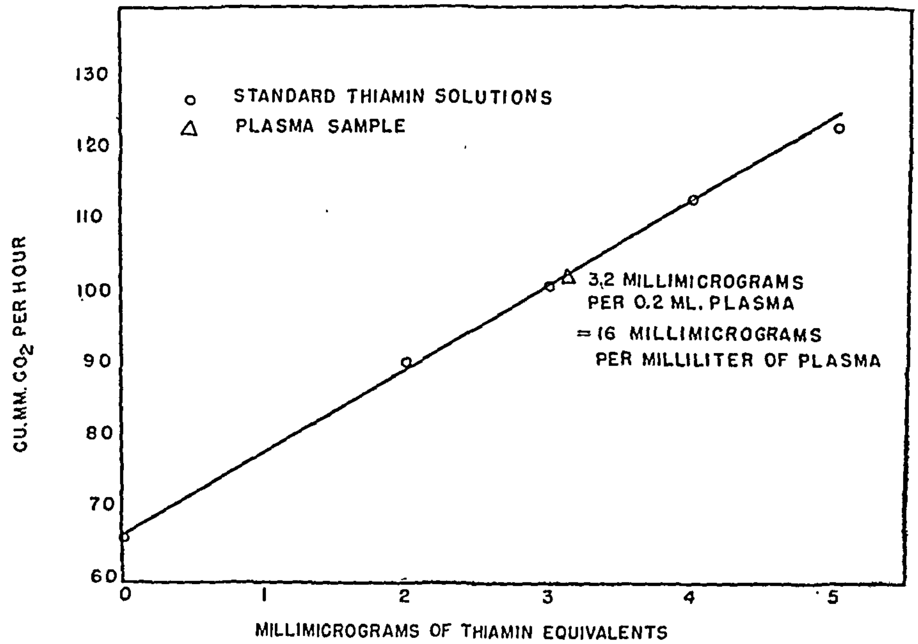

FIG. 1. C. MM. OF CO_2 PRODUCED IN ONE HOUR BY STANDARD THIAMIN SOLUTIONS

The plasma sample was diluted so that 1 ml. of the diluted plasma used equalled 0.2 ml. of the original plasma.

heparinized blood are acidified with 0.1 normal sulfuric acid to pH 4.5 and diluted with 1 to 4 volumes of water, the dilution factor varying with the estimated nutritional status of the patient. Duplicate or triplicate 1 cc. samples of the diluted and acidified plasma are incubated with special yeast suspensions in Warburg vessels (10), the carbon dioxide evolution measured, and the thiamin equivalence calculated from standard curves as previously described (15) (see Table I and Figure 1).

Correction for yeast-stimulating material, other than thiamin, in plasma is obtained by destroying the thiamin in duplicate aliquots of plasma with sulfite and determining the residual yeast-stimulating activity. Five cc. aliquots of plasma are acidified, sulfited, and heated as in the procedure described by Schultz, Atkin, Frey and Williams (13). This treatment, applied to plasma, results in the formation of a heavy protein coagulum, which is removed by suction filtration. The procedure for removal of excess sulfite from the solution (13) is then completed on the clear filtrate, the acidity adjusted to pH 4.5, and 1 cc. samples incubated, as described above. By difference, the "true" thiamin content of plasma can then be calculated.

The coagulum of plasma proteins is a probable source of error in this procedure. Up to the present time, we have not found suitable conditions under which sulfiting may be conducted without the precipitation of protein. On the basis of our experience, we estimate that there is introduced an error of 20 per cent, due to adsorption of yeast-stimulating material on the coagulum.[3]

[3] This investigation was terminated because of other war work.

RESULTS

A number of experiments were performed to assay the stability of the yeast-stimulating materials in human plasma. The stability in contact with red cells, that is, in heparinized whole blood, was first investigated.

Plasma that was separated immediately from a sample of freshly drawn, heparinized, whole blood had a yeast-stimulating activity equivalent to 8.0 millimicrograms of thiamin per cc. of plasma. Plasma, separated after the heparinized whole blood had stood for 30 minutes at room temperature, had a yeast-stimulating activity equivalent to 7.8 millimicrograms of thiamin per cc. of plasma. It would therefore appear unnecessary to work with more than moderate expedition in collecting samples of blood for plasma thiamin analyses.

The stability of the yeast-stimulating materials in plasma, after removal of the blood cells, was next determined. The activity of untreated plasma was found to decrease on standing in the refrigerator. The activity of samples, acidified to pH 4.5 with 0.1 normal sulfuric acid, remained constant for periods of several days (Table II).

The greater portion of the yeast-stimulating

material of plasma appeared to be freely diffusible at pH 5 to 7, in other words, probably free to exchange between plasma and general extracellular fluid. Five cc. samples of plasma were dialyzed in cellophane sacs against 200 cc. of 0.9 per cent sodium chloride, at pH 5, 6, and 7, for periods of 20 hours, at 5° C. By this period of dialysis, 86 ± 6 per cent of the plasma activity was removed (Table III).

The plasma of subjects who are in the habit of taking vitamin tablets containing thiamin was found to have a higher yeast-stimulating activity than that observed in the usual normal individual. The yeast-stimulating activity fell to normal ranges promptly upon the cessation of thiamin medication and became less than normal in individuals subsisting upon an experimental diet deficient in thiamin (16) (Table IV).

In a heterogeneous group of samplings (Table V), the "true" thiamin content of plasma was found to range between 1.7 and 13.5 millimicrograms per cc., the variations appearing in gross coordination with the thiamin nutrition of the subjects. Individuals known to be taking vitamin tablets containing thiamin, or to have recently ingested food of high thiamin content, seemed to have higher plasma thiamin values than subjects whose thiamin nutrition was less generous. In this connection, the low value of 1.7 millimicrograms of thiamin per cc. of plasma observed in the last subject, T, is interesting. This individual, a young woman in good physical condition, had been told independently by an outside physician that she was "lacking in B vitamins." This diagnosis had been made on the basis of a glossitis with moderate atrophy of the marginal papillae and hypertrophy of the fungiform papillae.

The blanks, or plasma activities after sulfiting, are given in Table V. As can be seen in this

TABLE II

Effect of pH upon the preservation at 5° C. of the yeast-stimulating activity of human plasma

Activity expressed in thiamin equivalents, millimicrograms per cc. of plasma

Period of preservation	pH of preservation	Yeast stimulating activity
days		*millimicrograms*
0		6.5
1	7.4	5.3
0		6.6
1	7.4	4.1
0		6.1
2	4.5	6.0
4	4.5	6.0
0		5.7
5	4.5	5.9

TABLE III

Diffusibility of yeast-stimulating materials of human plasma dialyzed against normal saline at pH 5, 6, or 7

Yeast-stimulating activity expressed in thiamin equivalents, millimicrograms per cc.

Sample	pH of dialysis	Thiamin equivalents before dialysis	Thiamin equivalents after dialysis	Per cent removed by dialysis
W	7	13.2	1.7	87
W	6	16	3.2	80
W	6	7.5	0.8	89
N	7	8.8	1.2	87
N	6	8.8	0.7	92
H	6	8.3	1.0	87
H	5	8.3	.7	91

TABLE IV

Yeast-stimulating activity of plasma of individuals of varying nutritional status

Activity expressed in thiamin equivalents, millimicrograms per cc. of plasma

Subject	Sex	Nutritional status	Plasma activity
P	F	Normal diet plus 6 vitamin tablets o.d.	16.0
W	M	Normal diet plus 3 vitamin tablets o.d.	13.2
W	M	Normal diet plus 3 vitamin tablets o.d.	16.0
W	M	Normal diet without vitamin tablets for 1 week	7.5
W	M	Normal diet without vitamin tablets for 1 month	8.5
B	M	Normal diet plus 2 yeast cakes o.d.	9.3
B	M	Normal diet without yeast for 1 week	7.3
N	M	Normal diet	8.8
H	F	Normal diet	8.3
R	F	Normal diet	6.9
T	F	Usual diet (? thiamin deficient)	5.0
Bt	M	Thiamin deficient diet, 1 week. Usual exercise	4.1
S	M	Thiamin deficient diet, 1 week. Walked 15–20 miles a day	2.5
Ko	M	Thiamin deficient diet, 2 weeks. Usual exercise	3.9
Ki	M	Thiamin deficient diet, 2 weeks. Walked 15–20 miles a day	2.9

table, the blanks fluctuate in a narrow range from 2.4 to 5.5 millimicrograms. Since thiamin deficient plasma activity is almost wholly due to blank substances, the importance of determining the blank value is obvious. The data available at present are insufficient to evaluate the relationship of the blank to normal, subclinically deficient, or deficient plasma thiamin.

Comparison of the yeast-stimulating activity of plasma and the 24-hour urinary excretion of thiamin by the thiochrome method (17) reveals a rough parallelism between these two measurements of thiamin nutrition (Table VI). The quantitative aspect of the parallelism is interesting in that it affords some information on the physiology of thiamin excretion. Thus, in a fasting individual, the glomerular filtration rate for plasma was found by mannitol clearance [4] (18) to be 76 cc. per minute. The thiamin excretion at the same time was 130 millimicrograms per minute. The yeast-stimulating activity of the plasma during the experiment was equivalent to 5.6 millimicrograms of thiamin per cc. of plasma. Assuming a plasma blank of the order of 3, the theoretical glomerular excretion of thiamin might be calculated as (5.6–3) $\times$ 76 or 198 millimicrograms per minute. This calculation, while inaccurate because of the assumption of the size of the plasma blank, appears to preclude the possibility of an extensive tubular reabsorption of thiamin.

TABLE V

Yeast-stimulating activity of human plasma before and after treatment with sulfite

Activity expressed in thiamin equivalents, millimicrograms per cc. of plasma

Sample	Activity of plasma before sulfiting	Activity of plasma after sulfiting	"True thiamin"		Nutritional status
			mμ* per cc. of plasma	Percentage of total activity	
P	16.0	2.5	13.5	84	Normal diet plus 6 vitamin tablets o.d.
B	12.2	2.4	9.8	80	Normal diet (sample taken 1 hour after noon meal)
H	10.6	3.0	7.6	72	Normal diet
L	10.0	4.4	5.6	56	Normal diet (1 vitamin tablet on previous evening)
W	8.5	5.5	3.0	35	Normal diet
T	5.0	3.3	1.7	34	Usual diet (? thiamin deficient)

* 1 mμ equals 1 millimicrogram.

[4] We are indebted to Dr. W. C. Bridges, Peter Bent Brigham Hospital, Boston, for this measurement.

TABLE VI

Comparison of the yeast-stimulating activity of plasma and the 24-hour urinary excretion of thiamin

Yeast-stimulating activity expressed in thiamin equivalents, millimicrograms per cc. of plasma

Subject	Yeast stimulating activity of plasma	Thiamin content of 24-hour urine sample	Nutritional status
	millimicrograms	*micrograms*	
W	16.0	500	Normal diet plus 3 vitamin tablets o.d.
B	9.3	190	Normal diet plus 2 yeast cakes o.d.
W	8.5	125	Normal diet without vitamin tablets for 1 week
B	7.3	100	Normal diet without yeast for 1 week
Ko	3.9	41	Thiamin deficient diet for 2 weeks, usual exercise
Ki	2.9	27	Thiamin deficient diet for 2 weeks, walked 15–20 miles o.d.

DISCUSSION

Evidence has been presented to indicate that measurements of plasma thiamin are technically feasible, using the yeast-fermentation method of thiamin assay (10, 15) and the sulfite cleavage method of distinguishing thiamin from other materials having yeast-stimulating activity (13).

Dialysis experiments have indicated that a major portion, at least 80 to 90 per cent, of the yeast-stimulating material of plasma is freely diffusible at pH's 5 to 7 and hence, *in vivo*, probably is in free exchange with similar material, notably thiamin in the general extracellular fluids. This observation is important in lending support to the idea that changes in plasma thiamin concentration may be taken as an index of changes in the thiamin content of the general cellular environment.

Since the functional thiamin enzymes are, on the whole, intracellular components of considerably greater concentration than that obtained by the thiamin of the extracellular fluids (ratio about 100 to 1) (5) and since it is inadequacy of these intracellular enzymes which, in all

probability, determines the symptoms of thiamin deficiency, it would be desirable to ascertain to what extent variations in plasma thiamin concentrations may be indicative of changes in intracellular thiamin concentrations (10). Preliminary experiments to this purpose have been performed (16). From the observations reported in this paper, it is evident, however, that plasma thiamin concentrations, and, for that matter, total plasma yeast-stimulating activities, are quickly responsive to changes in thiamin nutrition, the responses being in many ways similar to those observed in urinary thiamin excretion. Thus, the plasma thiamin level, like the urinary thiamin excretion, appears to have a large degree of freedom in the range above the level of clinical thiamin deficiency, rising to several times normal value under generous thiamin administration and falling rapidly to subnormal levels with degrees of thiamin deprivation scarcely sufficient to produce signs or symptoms of thiamin deficiency.

SUMMARY AND CONCLUSIONS

(*1*) The yeast-stimulating activity of plasma was determined by the yeast-fermentation method and recorded in terms of thiamin equivalence.

(*2*) The yeast-stimulating activity due to thiamin in the plasma was destroyed by sulfite cleavage and the residual yeast-stimulating activity determined.

(*3*) The "true" plasma thiamin was calculated from (*1*) and (*2*) by difference.

(*4*) It was found that the yeast-stimulating materials in plasma, thiamin and others, were freely diffusible at pH's 5 to 7, from which it was concluded that they were probably free to exchange *in vivo* with similar materials in the general extracellular fluids.

(*5*) The yeast-stimulating activity of plasma was found to vary with the level of thiamin nutrition, with the level of thiamin excretion, and with the "true" plasma thiamin concentration.

(*6*) It was therefore concluded that measurements of the yeast-stimulating activity of plasma could be used as indices of thiamin nutrition. However, since some variation in activity, due to materials other than thiamin, was observed, calculation of "true" plasma thiamin appeared preferable, particularly in plasma of low thiamin content.

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CHARACTERISTICS OF THE NORMAL ELECTROENCEPHALOGRAM. I. A STUDY OF THE OCCIPITAL CORTICAL POTENTIALS IN 500 NORMAL ADULTS [1]

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(Received for publication August 2, 1943)

This study represents an attempt to codify the main characteristics of the electroencephalogram in the normal adult. This attempt was inspired by the recent interest in the use of the electroencephalogram as a possible method of grading normals in the selection of air pilots.

The chief characteristics of the normal electroencephalogram may be studied by considering the following components:

(*1*) *Dominant frequency* . . . which is here defined as the frequency in cycles per second of the majority of the waves present. The degree of fluctuation in the dominant frequency of an individual in repeated recordings has also been studied.

(*2*) *Percentage time alpha* . . . the percentage of the record occupied by waves of 8.0 to 13.0 cycles per second, whether occurring singly or in chains.

(*3*) *Percentage time intermediate frequencies* . . . the percentage of the record occupied by waves in the intermediate band (13.5 to 17.5 per second).

(*4*) *Percentage time beta* . . . the percentage of the record occupied by cortical potentials of frequencies above 17.5 per second, and of voltages so low as to make them individually uncountable.

(*5*) *Percentage time slow activity* . . . *i.e.*, waves slower than 8.0 cycles per second.

(*6*) *Voltage* . . . In this study, the voltage characteristic studied was the maximum voltage of the potentials from the bipolar occipital leads.

The characteristics listed above will be found to vary in the same person, according to the part of the head examined. Throughout the present study, all analyses were made from bipolar recordings from the occiput.

[1] This study was aided by a grant from the Harrington Fund.

METHOD OF ANALYZING THE RECORDS

After many attempts at easier and more rapid methods of analysis, the method finally chosen for this research, because it gave more information than any other, was the frequency distribution of the waves, compiled by counting the percentage time covered by waves of each different number of cycles per second.

In order to compile a frequency distribution curve, a 2-minute record, taken when the subject was lying quietly and breathing normally, is first inspected for the presence of artifacts. Any portion showing artifacts due to eyeblinks, muscle movements, etc., is omitted from the sample for analysis. The remainder is measured for total length of time, and this figure becomes the total on which all percentages are calculated.

A transparent grating (designed by Davis), marked off in intervals equivalent to each of the frequencies, is then laid on the record, and the frequency of any chains of waves is thus easily determined. The time covered by waves of each frequency is then totalled, the results being expressed as percentages of the whole period measured.

This process can be shortened by measuring only chains in which at least 3 waves of the same frequency occur together; in the majority of normal records, this arbitrary rule gives an adequately representative picture of the record, although its only specific merit is as a time saver.

When these figures have been compiled, they can be presented either in tabular form, or in diagrammatic form (Figure 1). On the whole, the diagram is to be preferred, because it gives an immediate representation of the most characteristic features of the electroencephalogram.

The characteristics listed in the introduction were studied in detail in a series of 500 young normal adults and the records of these subjects form the basis for the major portion of this paper. Most of these subjects received only one test, but in order to study the consistency of various of the properties of the electroencephalogram, 45 of the series were given 4 to 5 tests each; in all, 176 tests were made on this smaller series of 45.

RESULTS

(*1*) *Dominant frequency*

(*a*) *Types of dominant frequency.* On first inspection, the most outstanding feature of an electroencephalogram is the dominant rhythm,

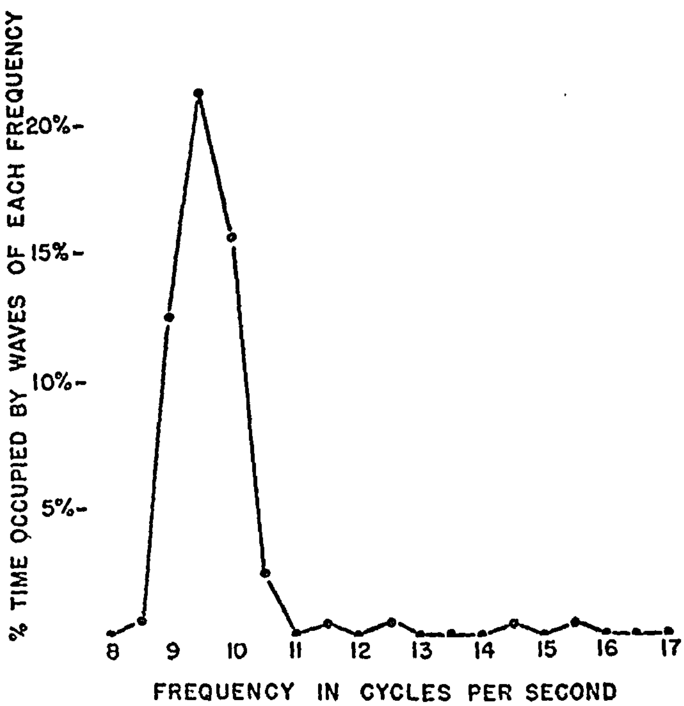

FIG. 1. GRAPH COMPILED FROM THE ELECTROENCEPHALOGRAM OF ONE NORMAL ADULT SHOWING THE PERCENTAGES OF EACH FREQUENCY PRESENT, OMITTING THE BETA RANGE

The dominant frequency is defined as the one present in the greatest amount, *i.e.*, at the mode of the curve. (In this case, it is 9.5 cycles per second.)

i.e., the frequency in cycles per second which is present in greater amounts than any other frequency. This frequency is usually apparent on rough inspection and most electroencephalographers have adopted the classification into two groups, as first suggested by Berger, namely, the alpha and the beta types. These have usually been defined as the frequencies between 8.0 and 13.0 cycles per second for the alpha group, and frequencies faster than 18.0 cycles for the beta group. This classification is not satisfactory since, in fact, all electroencephalograms consist of a mixture of these rhythms in some degree, and no record consists wholly of either alpha or beta waves. Hence, if this classification is to be used, some criterion must be defined as to the percentage of beta activity which must be present in a record before that record should be classified as a beta type. For the purposes of the present study, records are classed as beta type only if there is present less than 20 per cent of other activity, *i.e.*, of waves slower than 18 cycles per second.

Also, in the present work, a third classification is used, since it has been found in this series of 500 normal adults that there is evidence for regarding the dominant frequencies in the intermediate band (13.5 to 17.5 cycles per second) between the alpha and the beta ranges as a separate entity (Figure 2).

A detailed statistical analysis was made of the activity slower than beta (*i.e.*, slower than 18.0 cycles) in the records of 500 normal adults. The mean for all the dominant frequencies in this range was 10.5 cycles per second, with a standard deviation of 0.9. Thus, any dominant frequency slower than 8.0 or faster than 13.0 cycles is outside 3 times standard deviation for normals, and is therefore, by definition, excluded from the alpha range. Further reason for regarding records with a dominant frequency in the intermediate range as a separate group is found in a study of the distribution curve of the dominant frequencies of 500 normal adults (Figure 3).

In this graph, there appears to be a normal distribution curve dominating the picture but with some outlying stragglers in the faster frequencies. The main curve consists of 474

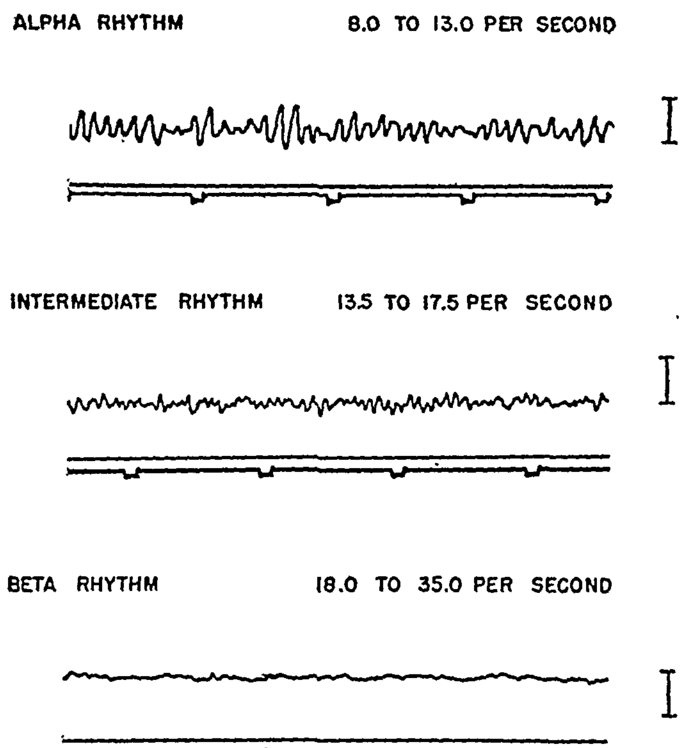

FIG. 2. TYPES OF NORMAL ELECTROENCEPHALOGRAMS

The upright line to the right of each tracing is the calibration for 100 mV.

subjects whose dominant frequencies give a normal distribution around a mode of 10.0 cycles per second, with 26 subjects outside the normal distribution curve. Hence, it is felt that those frequencies in the range 13.5 to 17.5 should not be included in the alpha group. Frequencies falling within this range are referred to as intermediate rhythms.

In this group of 500 normal adults, examined in this laboratory, the following distribution of the three types (alpha, intermediate, and beta) was found:

Number of subjects:	500	*per cent*
Number of alpha type (8 to 13.0 cycles per second):	474	94.8
Number of intermediate (13.5 to 17.5):	18	3.6
Number of beta type (18.0 and over):	8	1.6

(*b*) *Consistency of the dominant frequency.* That the dominant frequency remains constant within narrow limits for the same individual over long periods of time can be demonstrated by repeated observations on the same person. A discussion of some factors which may, in certain circumstances, alter the dominant frequency will be reported in a subsequent paper.

Electroencephalograms repeated on the same subjects over a period of a few years revealed only small fluctuations in the dominant frequency from one test to the next:

Subject 1, aged 35, female	
July 1940	9.5
Dec. 16, 1940	9.5
Dec. 26, 1940	9.5
Sept. 1942	9.5

Subject 2, aged 24, female	
Oct. 1940	9.5
June 1941	10.0
April 1942	10.0
Sept. 1942	10.0

Subject 3, aged 36, female	
Dec. 1940	20.0
June 1941	20.0
July 1941	18.0
Aug. 1941	20.0

This degree of fluctuation is of the same order as that reported by other workers (Loomis, Harvey, and Hobart (1), Jasper and Cruikshank (2), and Jasper and Andrews (3)).

That there is also only a small fluctuation in the dominant frequency of an individual when examined several times during the same day, has been established on a larger group.

One hundred and seventy-six observations were made on 45 normal subjects, all of whom were examined at non-fasting blood sugar levels (above 70 mgm per 100 cc.), *i.e.*, 4 to 5 tests were made on the same individual at intervals during the same day.

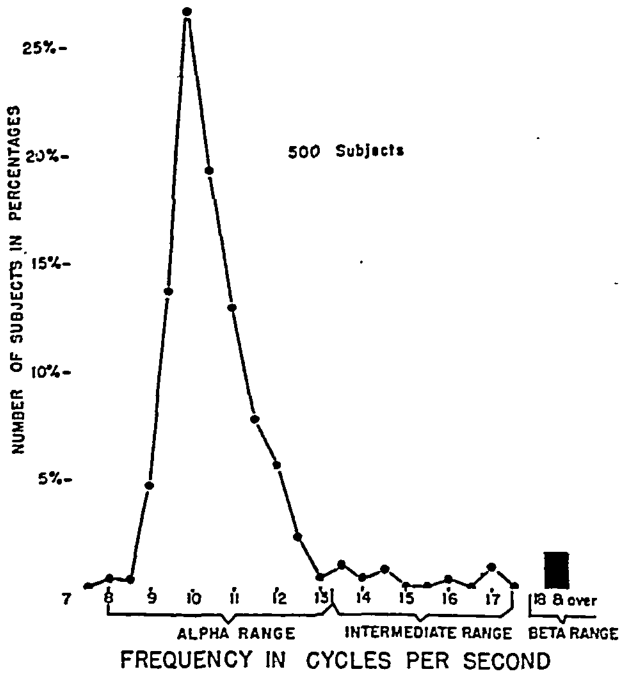

Fig. 3. The Distribution Curve of the Dominant Frequencies Found in the Occipital Rhythms of 500 Normal Adults

Ten of the 45 subjects showed no variation in their dominant frequency. In 1 subject only did the dominant frequency vary from his own mean value by more than 7 per cent. The mean variation for the whole series of 45 subjects was under 1 per cent (176 observations).

(*c*) *Relation between consistency of the dominant frequency and age.* If the consistency of a person's dominant frequency be studied, it is found that age is a factor in the degree of variability found in the electroencephalogram at non-fasting blood sugar levels (above 70 mgm).

This degree of variability is determined by finding the coefficient of variation for the dominant frequency of each individual. $\left(\text{The coefficient of variation} = 100 \times \frac{\text{standard deviation}}{\text{mean}}.\right)$

A group of 45 young adults between the ages of 17 and 38 were thus examined for consistency of dominant frequency at normal blood sugar levels. By rough observation, it appeared as though the dominant frequency in records of those subjects over the age of 20 was less stable than of those under that age, and for this reason the division into two groups was made at this age level. In the series examined, there were

13 subjects under the age of 20, and 32 were aged 20 or over. Calculations of the coefficient of variation gave the following result:

	Under 20	20 and over
Mean of coefficient of variation	1.2	3.2
Standard deviation of coefficient of variation	1.3	2.4

The difference between these two means was tested for reliability by determining the standard error of the difference:

$$\frac{D}{\sigma D} = 3.52,$$

where D equals the difference between the two means, and σD, the standard error of that difference.

The result is indicative of a significant difference between these two groups, *i.e.*, the chances of this being a true difference are over 1000 to 1.

It would appear therefore that the dominant cortical frequency becomes less stable with increasing age, and this is demonstrable even in a series which contains no one over the age of 38.

(*d*) *Dominant frequency and the age factor.* The total group of 500 young adults, between the ages of 17 and 47, was studied for correlation between their age and the actual frequency of their dominant rhythm at non-fasting blood sugar levels (in contrast to the consistency of this dominant frequency which has just been examined).

There have been several studies of this kind in relation to age in children (Berger (4), Loomis (5), Lindsley (6), Smith (7), and Weinbach (8)); but the age factor in adults has not received much attention. Bernhard and Skoglund (9, 10) demonstrated a difference of over half a cycle in the mean dominant frequency between two age groups of 15 to 18 and 19 to 30, respectively.

In the present series of 500 adults between the ages of 17 and 47, with a mean age of 24, the following results were obtained:

Under 24 years

270 alphas—mean dominant frequency 10.5
7 intermediates
4 betas

24 years old and over

204 alphas—mean dominant frequency 10.4
17 intermediates
4 betas

Thus, it would appear that age has no influence on the dominant frequency of the electroencephalogram of adults up to the age of 47. We have no data on normal individuals over this age.

(*e*) *Dominant frequency and its relation to other physiological factors.* The dominant frequencies in this group were examined for any possible correlation with sex, weight, height, or height-weight ratio, but no relation was found with any of these factors.

(*2*) *Percentage time alpha*

This is defined as the percentage time occupied by waves of *8.0 to 13.0 cycles per second*, occurring either singly or in chains; *i.e.*, it is the gross alpha count.

In 500 subjects who were examined at non-fasting blood sugar levels, the percentage time alpha varied in the group from 9 to 93 per cent, with a mean of 61 per cent. There was some alpha activity present in all records, even those which were predominantly beta in type.

The distribution among this group of the amount of alpha activity present at non-fasting blood sugar levels is given in the following distribution diagram (Figure 4).

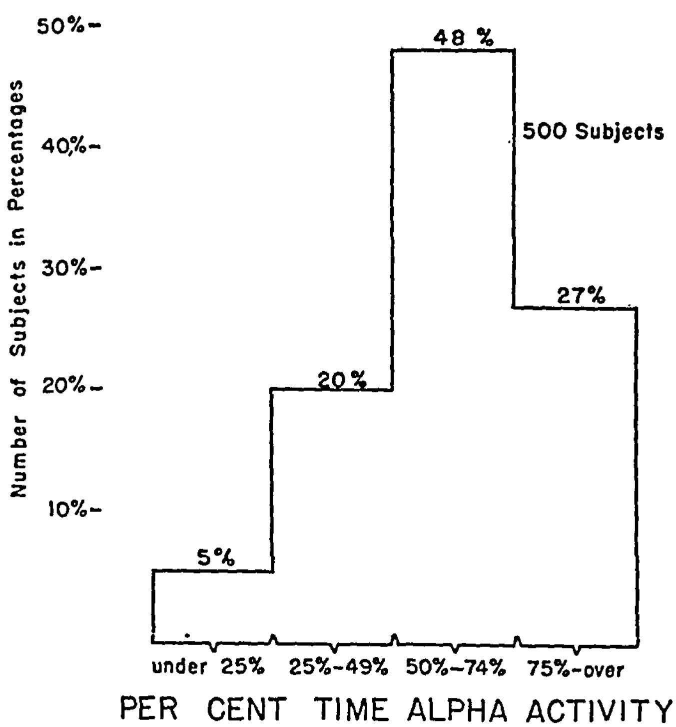

FIG. 4. GRAPH ILLUSTRATING 500 NORMAL SUBJECTS (EXPRESSED AS PERCENTAGES) GROUPED ACCORDING TO THE AMOUNT OF ALPHA ACTIVITY PRESENT IN THEIR RECORDS

Of the 24 subjects with less than 25 per cent alpha activity, 16 had a predominantly intermediate rhythm, and 8 had dominant frequencies in the beta range.

(*a*) *Consistency of percentage time alpha.* The variability in percentage time alpha for an individual was not so marked in this series as has been described by Rubin (11) in bipolar recordings, the mean standard deviation for repeated tests on an individual being 7.1. It should be pointed out that in this work the percentage time alpha quoted includes all alpha activity present, whether occurring in single waves or in chains, whereas Rubin's observations are based on a criterion of 3 waves of alpha frequency occurring together; and in Rubin's experiments, the blood sugar was not controlled.

The relation between variability of percentage time alpha and high or low alpha percentage, as described by Rubin (12), did not hold in this series.

(*b*) *Relation between percentage time alpha and dominant frequency.* There was found to be an inverse relationship between percentage time alpha and the dominant frequency of that alpha; in other words, individuals whose percentage time alpha values were high showed dominant frequencies in the slower alpha range.

In the total series of 500 subjects, the records were examined for the relation between the dominant frequency of any alpha present and the percentage time occupied by this alpha activity. The following results were obtained:

Alpha frequency	Number of subjects	Mean percentage time alpha	Standard deviation
Slower than 10.5 cycles	236	68.5	14.1
10.5 cycles and faster	264	54.7	20.1

The difference between these 2 means was tested for reliability by determining the standard error of the difference which was found to be 8.90. Such a high standard error of the difference is beyond the possibility of chance.

It is therefore concluded that an inverse relationship exists between the dominant frequency of the alpha present and the amount of total alpha present.

(*c*) *Relation between percentage time alpha and other physiological factors.* No correlation was found in this series between the percentage time alpha and age, sex, height, weight, or the height-weight ratio.

(3) *Percentage time intermediate rhythm*

Frequencies of 13.5 to 17.5 cycles per second, *i.e.*, those which lie between the alpha and beta ranges, are not commonly found in more than negligible quantities in the records of normal adults, and are only rarely found as the dominant frequency of the record. In this series of 500 subjects, there were 18 with a dominant frequency in this range, or 3.6 per cent.

Rhythms in this range, however, normally occur in bursts during the lighter stages of sleep (13), and it would not be surprising to find that they have a different physiological origin from the alpha waves. They do not appear to be merely accelerated alpha waves, since observations on sleep show that they appear abruptly and do not emerge by gradual transition from the higher alpha frequencies. These facts suggest that one is here dealing with a dichotomy.

As has already been noted, the electroencephalogram in normal adults contains very little activity in this intermediate frequency band. What little there is might be expected to appear in those records with the faster alpha frequencies as an extreme variation of their predominantly 12.5 to 13.0 cycle rhythms, but an examination of this series failed to establish any such correlation. There were no more waves of the intermediate frequencies in those records with dominant frequencies in the faster alpha range than in those with predominantly 9.0 and 9.5 cycle activity.

Unlike the so-called alpha and beta rhythms which are present to some extent in all records, the intermediate band of 13.5 to 17.5 cycle activity is sometimes totally absent, a fact which contributes to the impression, previously mentioned, that one is here dealing with a dichotomy.

(4) *Percentage time beta*

Beta activity (*i.e.*, 18.0 cycles per second and over) was found, to a greater or less extent, in every record in this series at non-fasting blood sugar levels. As has already been remarked, all

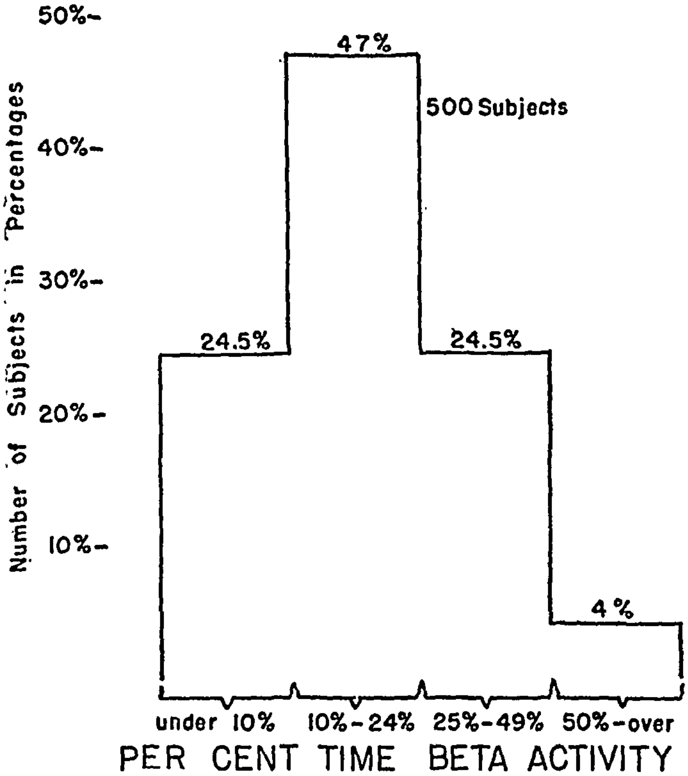

FIG. 5. GRAPH SHOWING 500 NORMAL SUBJECTS (EXPRESSED AS PERCENTAGES) GROUPED ACCORDING TO THE AMOUNT OF BETA ACTIVITY PRESENT IN THEIR RECORDS

electroencephalograms are a mixture of rhythms, and, in this series, the 8 cases which have been classified as beta type, all contained some alpha; their percentages of beta activity varied from 51 to 80 per cent. The distribution of beta activity in this series is given in Figure 5.

Figures 4 and 5 taken together do not, of course, give the total picture for the group, since the time occupied by intermediate frequencies and by slow activity is not represented. The amount of beta activity in an individual's record was found to be remarkably constant in repeated runs, and is characteristic for the individual. It did not vary with the degree of relaxation achieved by the subject.

Although in this series there was a tendency to more beta activity in the records of the older subjects, no statistical relation between it and age could be established. The amount of beta activity present did not correlate with sex, height, or weight.

(5) *Activity slower than 8.0 cycles per second*

Waves of a frequency slower than 8.0 cycles per second are here referred to as slow activity. Traces of 7.0 to 7.5 per second frequencies were found in the records of 125 out of the series of 500 subjects, but only in 4 individuals was there more than 5 per cent of such slow activity in the 2-minute recording; of these 4 individuals, 1 had 19 per cent slow activity, 2 had 9 per cent, and 1 had 6 per cent.

Six-cycle waves were found in the occipital leads in 41 (8 per cent) of the 500 records at non-fasting blood sugar levels, but waves slower than 6 cycles per second were found in only 4 subjects, or less than 1 per cent.

Were an investigation of the potentials from the temporal regions to be made in a way similar to the present detailed study of the occipital potentials, it seems likely that there would be a higher incidence of 6- and 7-cycle waves in normal records, this being our experience and that of other electroencephalographers (14).

No wave in the range commonly called delta activity (*i.e.*, 4 cycles or slower) was found.

(6) *Voltage*

This was measured by a pair of calipers, adjusted to the calibration made for voltage at the beginning of each record.

The maximum voltage was measured in every case and classified as to whether it was under 25 mV, over 25 mV but under 50 mV, over 50 mV but under 100 mV, or over 100 mV. In the latter case, a further breakdown in classification was made between those who had less than 20 waves which reached 100 mV in amplitude and those which had more than this number in a 2-minute run.

The distribution of voltage in this series of 500 normal subjects at non-fasting blood sugar levels was as follows:

Total	Under 25 mV	Over 25 mV but under 50 mV	Over 50 mV but under 75 mV	Over 75 mV but under 100 mV	Over 100 mV
500	16	109	151	128	96

(*a*) *Voltage and dominant frequency*. The faster frequencies tend to be of low voltage; no record with a dominant frequency faster than 11.5 had any potentials which reached as much as 100 mV.

In the alpha range, the records with the slower alpha frequencies were of higher voltage than

the faster ones, thus following the usual character of oscillations in which the amplitude is inversely proportional to the frequency. In the following table, 474 normal subjects, whose dominant frequencies were in the 8.0 to 13.0 cycle band, are listed according to their voltage.

	Mean dominant frequency
Maximum voltage under 50 mV (105 subjects)	11.0
Maximum voltage over 50 mV (274 subjects) but under 100 mV	10.4
Maximum voltage over 100 mV (95 subjects)	10.0

(*b*) *Voltage and percentage time alpha.* The maximum voltage of the potentials in a record vary directly with the percentage time alpha activity present; *i.e.*, those records which have a large amount of 8.0 to 13.0 cycle waves reach a higher maximum potential.

In the following table, voltage is related to the mean percentage time alpha found in the same series of 474 normal subjects whose dominant frequencies were in the 8.0 to 13.0 cycle band.

Maximum voltage	Mean percentage time alpha
Under 25 mV	40.3 (10 subjects)
Over 25 mV, under 50 mV	48.4 (95 subjects)
Over 50 mV, under 100 mV	65.4 (274 subjects)
Over 100 mV	73.6 (95 subjects)

DISCUSSION

Since the first development of electroencephalography, interest has been centered mainly on its application to clinical problems, and it is only recently that there has been a shift of interest to the study of the normal adult. For many reasons, it would have been preferable had the reverse taken place, for the development of this test to have proceeded from a basic study of the normal to a comparison of clinical records with a norm already well established.

The desirability is patent for the establishment of a yardstick for the normal population against which may be measured the variables found in pathological records. It is equally desirable in attempting to assess the electroencephalograms of normal subjects (as, for example, is being done in air-pilot selection) to have a quantitative basis from which one may calculate the chances of any observed phenomenon being a normal finding.

In this paper, a beginning has been made in an attempt to find what range of variation can be found for some of the characteristics of the electroencephalogram of normal adults. At the present stage, this study has been limited to an analysis of the cortical potentials from the occipital lobes, and it cannot be too strongly emphasized that a different set of data would undoubtedly be obtained from the frontal lobes, and different again from the temporal and parietal regions.

This study is also limited to analysis of electroencephalograms during normal breathing. A report of an investigation during hyperventilation will follow this, with special reference to the rôle of blood sugar and depth of hyperventilation.

In the past, the bulk of the work on both normal and clinical electroencephalograms has been done by the method of appraisal. The experienced electroencephalographer has looked at the record, compared it in his mind with his impression of those records which have previously come into his laboratory, and assessed it from this mental comparison. Where gross differences are present, such as are found in patients with epilepsy or with neoplasms, this method has in the main sufficed, but when finer shades of difference are being searched for, a more finely differentiated set of standards is necessary.

Such standards can only be set up on a basis of actual measurement, a method which is time absorbing, but essential in any research project designed to establish normal control standards. Were such a set of standards established on a large enough group of individuals, it would then be possible to estimate the chances of normality when any fine differences occur in the record, as for example, 7 cycle waves occurring singly in the occipital leads, or trains of 14 cycle waves. The percentage of normal records in which such waves occur would be known and the importance of the finding could thus be assessed.

A development in the measuring of electroencephalograms has been made by Gibbs and Grass (15) in the form of a spectrum analyzer. This apparatus gives a compilation of the amount of energy present at each frequency. At the present stage of our experience, we have found more meaning in the frequency of waves than

in their voltage in normal records, and we therefore look for a method where the number of waves present at any frequency is not obscured by the voltage. An instrument for this purpose has recently been designed by Walter (16), but is not yet on the market.

It is obvious that the current differentiation of electrical potentials into alpha, beta, and delta rhythms is arbitrary and, in some respects, unfortunate. The classification of records into alpha and beta rhythms tends to obscure the fact that many so-called alpha records contain potentials of faster frequencies and that many beta records contain percentages of the slower alpha frequencies. A more accurate and more complete assessment could be made by describing the frequency distribution on the potentials. It would seem much wiser to describe rhythms in terms of the incidence of actually measured frequencies until some physiological or statistical reason can be found for grouping frequencies into certain rhythms.

The fact that significant contributions to clinical diagnosis in epilepsy and the localization of neoplasms have been made by the crude methods of gross inspection of records would by no means invalidate the need for studies based upon careful measurements. However, once the data on a sufficiently large number of cases are collected, it might well be possible to develop simpler methods of analysis, based upon the known verifiable distributions of frequencies. This would seem a more logical approach and could give more precise information in the study of problems in which fine differentiations occur in the records.

SUMMARY

The occipital cortical potentials have been analyzed under conditions of controlled blood sugar in 500 subjects. Of these, the majority received but one test, but in 45, repeated observations were obtained.

The following characteristics were found in these electroencephalograms.

1. The dominant frequency of an individual is comparatively stable, but becomes less so with increasing age. There is a statistically significant correlation between age and stability of dominant frequency (45 subjects).

2. Waves of 8.0 to 13.0 cycles per second ("alpha") were present in all records examined. The percentage time occupied by alpha waves varied inversely with the frequency of the dominant frequency. This inverse relationship has been established statistically in 500 subjects.

3. Waves of 13.5 to 17.5 cycles per second ("intermediate") are rarely found in any quantity in normal records, and constitute the dominant frequency in only 3.6 per cent of all normals examined (500).

4. Waves of 18.0 cycles per second and faster ("beta") were present in all records examined (500). The percentage time occupied by waves in this range is nearly constant for an individual in repeated runs (45 subjects).

5. Waves slower than 8.0 cycles per second are found in occipital potentials of 25 per cent of normal subjects. Waves as slow as 6.0 cycles were found in only 41 out of 500 subjects.

6. No waves of 4.0 cycles per second or slower ("delta") were found in the occipital recordings of any normal subject while breathing normally (500 subjects).

7. Maximum voltages are higher in those records which contain the most alpha activity, and in those records whose dominant frequencies fall in the slower alpha frequencies (500 subjects).

8. The physiological factors of sex, height, weight, or the height-weight ratio did not correlate with any characteristic in the brain wave record.

All the electroencephalographic tracings for this research were recorded in the Brain Wave Laboratory of the Massachusetts General Hospital with the cooperation of the director, Dr. Robert S. Schwab.

The authors are indebted to Mrs. Frances Cooperstein and Miss Margaret Gray for technical help, and to Mrs. Mary Newell for analysis of the records.

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CHARACTERISTICS OF THE NORMAL ELECTROENCEPHALOGRAM. II. THE EFFECT OF VARYING BLOOD SUGAR LEVELS ON THE OCCIPITAL CORTICAL POTENTIALS IN ADULTS DURING QUIET BREATHING[1]

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Although the rôle of blood sugar level in the slowing of cortical rhythms during hyperventilation is now very generally recognized, its effect on the electroencephalogram during quiet breathing has not been fully studied in the normal human subject.

Gibbs, Williams, and Gibbs (1), using the spectrum analyzer, concluded from a series of 4 adult normals that between extreme limits, alterations of blood sugar level have no effect on the frequency of the brain waves at normal depths of ventilation. Lennox, Gibbs, and Gibbs (2) found an upper limit of 50 mgm. for the blood sugar level to affect the electroencephalogram during normal breathing.

Davis (3) made a more extensive study on 30 normals to whom injections of insulin were given in order to lower the blood sugar below fasting levels. She found that, within 20 minutes of the injection, there was a reduction in the alpha rhythm with the appearance of 8 cycle waves. Within 30 minutes of the injection, delta waves dominated the picture. Her study was made while the subjects were breathing normally. Hoagland (4, 5) and his co-workers made a study of the alpha rhythm in schizophrenics at blood sugars so low as to induce loss of consciousness.

The present study is an attempt to establish the influence of blood sugar level upon the electroencephalogram at stages of unimpaired consciousness in the normal subject.

METHOD

The subjects for these experiments were all young adults, mostly college students (29 males, 16 females), between the ages of 17 and 38. A brief medical history was taken and, in most cases, a brief physical examination was made before the experiment. Only those whose history indicated the absence of medical, neurological, and psychiatric disease, and whose physical examinations were within normal limits were included in the series. As a result of these brief preliminary examinations, the data on 15 individuals out of the 60 who volunteered were discarded from the series.

The routine procedure was as follows: The subject reported to the laboratory in the morning, fasting. He was asked to lie down for a period of at least 30 minutes during which time the history was taken, a brief physical examination made, and the scalp electrodes attached.

A sample of blood was drawn for the microdetermination of capillary blood sugar, and the subject was attached, by a mouth mask fitted with flutter valves, to a large spirometer with an open circuit for a period of approximately 8 minutes. He breathed outside air from the spirometer at the normal rate and depth of ventilation for a period of 2 minutes, and was then told to breathe as deeply as he could, inhaling and exhaling to the rhythm of a metronome clicking 30 times a minute. The depth of ventilation could be followed on the spirometer scale, and if the subject was not reaching the required depth for his body weight, he was urged to breathe deeper. (A study of the effects of the hyperventilation period will appear in a later paper in this series.)

After the 3-minute period of hyperventilation, the subject was told to breathe naturally, and another sample of blood was taken for the blood sugar determination, one minute after the hyperventilation period. Normal breathing was then continued for a period varying from 2 to 6 minutes, until the minute-respiratory volume was back to its base-line level.

The subject was disconnected from the spirometer and was asked to describe his subjective sensations during the test; he was then given a brief questionnaire designed to review the subjective sensations systematically.

This whole procedure was repeated within approximately 30 minutes, with the subject still in the fasting state. After another similar interval, an intravenous injection of saline was given as a control on the insulin injection to follow; this was identical in quantity and appearance with the insulin injection, and the subject was ignorant as to its nature and purpose. This control injection was to

[1] This study was aided by a grant from the Harrington Fund.

check on any possible rise in blood sugar, such as is sometimes found in normal persons when excited.

If, at fasting blood sugar levels, the electroencephalogram had shown no slowing of rhythm, an injection of insulin was given intravenously, the dose being calculated to give $\frac{1}{10}$ unit per kgm. ideal body weight. Two complete experiments were then run through, one immediately after the injection, and one approximately 25 minutes after it, this being the time for the maximum effect of insulin.

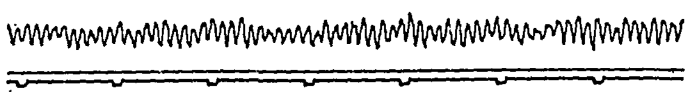
After this second run under the influence of insulin, the subject was given glucose by mouth, the usual amount being 100 grams of glucose in 100 cc. of water with lemon added to make it palatable. Three further experiments were then carried through, one immediately after ingestion of glucose, one 30 minutes after, and one approximately an hour later; if the electroencephalogram did not appear normal after the second of these runs after sugar, a second dose of glucose would be given. At the end of the hyperventilation period of each and every electroencephalogram recording, a blood sample was taken for blood sugar determination.

In all these experiments, the technique used for recording the electroencephalogram was standardized as follows:

Six scalp electrodes were applied in the usual manner to the right and left frontal, right and left occipital, and left parietal regions, respectively. In left-handed subjects, the parietal lead was put on the right parietal region. A reference electrode was placed on one mastoid process.

For the recording, a Grass electroencephalographic apparatus was used; in all the recordings, the paper speed was 3 cm. per second, and the pen excursion was calibrated for a sensitivity of 1 cm. for 100 mV. All recordings were made by remote control, the subject being in a darkened room at some distance from the laboratory where the electroencephalogram was recorded.

Electroencephalographic recordings were taken throughout the whole period of each experiment, except for the minute during which the subject was being pricked for the blood sample. These blood samples were analyzed for sugar content by a modification of the Folin-Wu micro method.



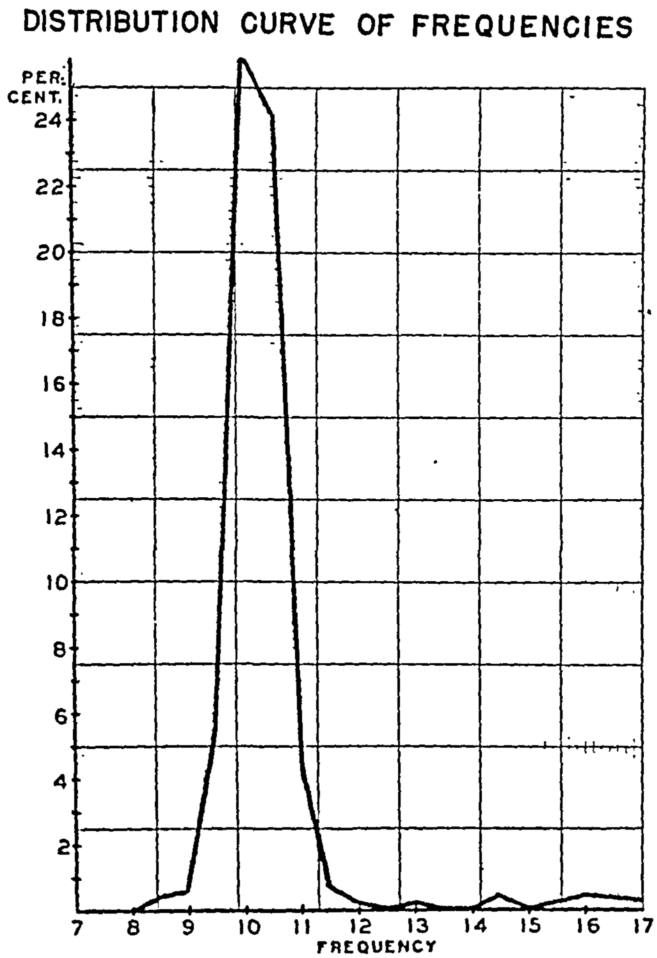


FIG. 1. DISTRIBUTION CURVE COMPILED FROM A NORMAL ELECTROENCEPHALOGRAM SHOWING THE PERCENTAGE OF EACH FREQUENCY PRESENT

The dominant frequency is defined as the one present in the greatest amount, *i.e.*, at the mode of the curve (in this case, it is 10.0 cycles per second).

RESULTS

The first characteristic of the electroencephalogram to be examined for the effect of low sugar was the dominant frequency, which is here defined as the frequency in cycles per second of the majority of the waves present.

This was determined by compiling a distribution curve of the frequencies found in the record from the occipital bipolar leads and was made by counting the percentage time occupied by waves of each frequency during the preliminary 2-minute run when the subject was breathing normally. A sample of one of these distribution curves is shown in Figure 1. The full technique, compiling these distribution curves of frequency, has been described in a previous paper (6).

The frequency at the mode of the curve, *i.e.*, the one present in the highest amount, is the one defined as the dominant frequency. This peak may fall in the alpha range (8.0 to 13.0 cycles), in the intermediate range (13.5 to 17.5), or in the beta range (18.0 or over).

Sixteen subjects with dominant frequencies in the alpha range received insulin injections, and these were examined for change in this frequency at low blood sugar levels. There were 2 to 3 recordings for each of these subjects at normal blood sugar levels (70 to 130 mgm. per

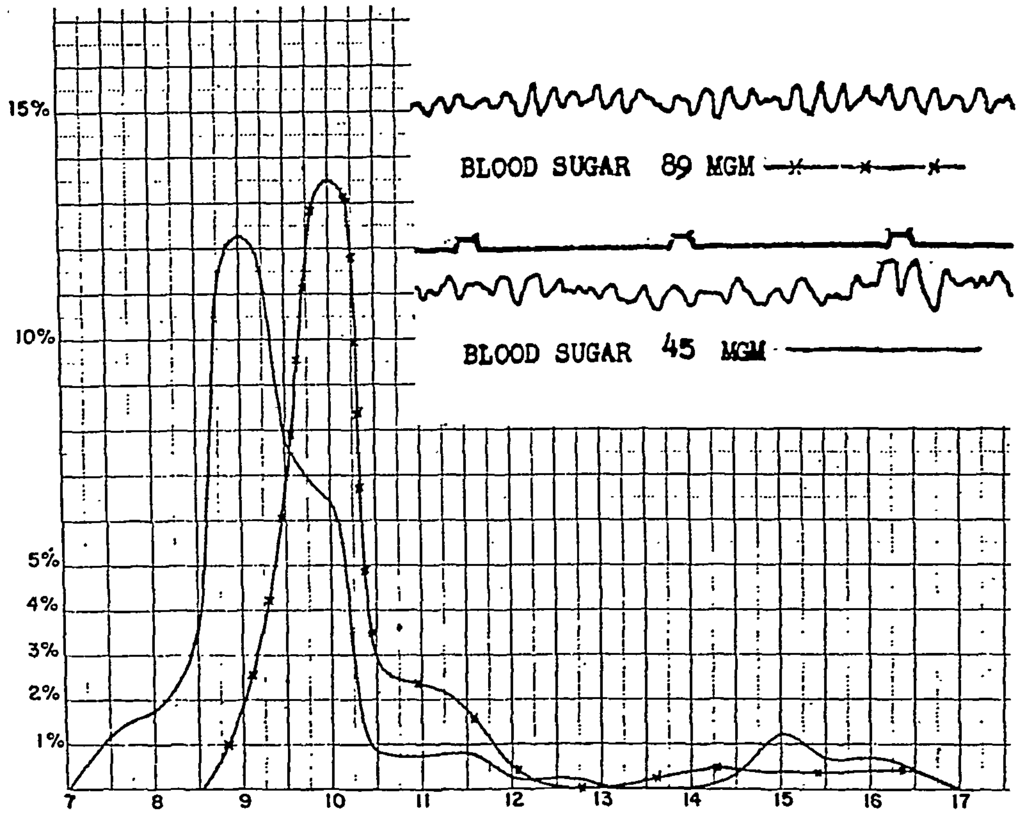


FIG. 2. DISTRIBUTION CURVES OF THE FREQUENCIES FOUND IN THE SAME INDIVIDUAL DURING A 2-MINUTE RECORDING WITH QUIET BREATHING AT TWO DIFFERENT BLOOD SUGAR LEVELS

100 cc.), so that a mean could be established as a base-line for each individual.

When these 16 individuals are examined as a group, it is found that the mean dominant frequency after insulin is slower than at ordinary sugar levels.

The mean dominant frequency after insulin injection differs from that at normal levels by more than 4 times the standard deviation at normal levels, and this may thus be regarded as a significant difference, and it can therefore be concluded that lowering the blood sugar below 70 mgm. per 100 cc. causes a slowing of the alpha rhythm during normal breathing. Blood sugar levels as low as 60 mgm. per 100 cc. are often found in fasting individuals who have received no insulin.

Sixteen subjects
(53 recordings)

Mean dominant rhythm at normal sugar levels (70 to 130 mgm.)	Probable error of this mean	Mean dominant rhythm 20 min. after insulin (under 70 mgm. blood sugar)	Probable error of this mean	Difference between the two means
10.56	0.132	10.00	0.257	0.56

An example of the distribution curve for one individual in the series (a male aged 21) is shown in Figure 2. This diagram gives the distribution of the various frequencies found at two different blood sugar levels in the same person while breathing at a normal depth.

The plain line is the curve for the blood sugar level of 45 mgm. and the crossed line is at 85 mgm. This is a chart of the alpha and intermediate ranges of activity, and does not represent the beta frequencies.

The effect of low blood sugar on the amount of slow activity during normal breathing

Waves of a rhythm slower than the alpha range (*i.e.*, slower than 8.0 cycles) are infrequently found in the potentials from the occiput

in the normal subject at ordinary blood sugar levels. In this series of 45 subjects, no one had as much as 1 per cent of slow activity, and 35 had none at all. In no case in this series were any waves slower than 6 cycles found.

Slow activity does appear in the records of some normal subjects if the blood sugar is lowered by insulin injection. Of 18 subjects who received insulin injections, 2 developed 6 cycle waves in occipital leads while breathing normally, and 2 others showed some 7.5 cycle. The maximum percentage time slow activity (*i.e.*, slower than 8.0 cycles) induced by insulin in any one in this series was 13.8 per cent at a blood sugar level of 45 mgm. per 100 cc.

The effect of high blood sugar (above 130 mgm.) on the dominant frequency

In this series of subjects, of those who were given glucose there were 31 (with alpha rhythms) whose blood sugars rose above 130 mgm. per 100 cc. Their mean dominant frequency was 10.31 which does not differ from the mean dominant frequency (*i.e.*, 10.33) found at ordinary blood sugar levels.

The practical outcome of this finding, coupled with that of the slowing of the rhythm at low blood sugar levels, is that in any assessment of an electroencephalogram on a basis of frequency, one can insure against changes due to low sugar by giving the subject glucose before the test, without any fear that changes may accrue from too high blood sugar.

Effect of high blood sugar on the percentage time alpha, intermediate, and beta rhythm

At blood sugar levels above 130 mgm. per 100 cc., as induced by glucose ingestion, there is no change in the electroencephalogram from the rhythms and patterns found at normal blood sugar levels.

DISCUSSION

This quantitative study of the frequencies of cortical potentials has shown that they may be slowed in the normal adult by moderate lowering of the blood sugar level without impairment of consciousness. Since the alpha rhythm has been shown, by Hoagland's (7) experiments with pyrexia, to be directly determined by the local respiration of the cells of the cortex, any deprivation of the oxygen supply to these cells would be expected to slow the rhythm. Dextrose being the principal substrate in cerebral metabolism, hypoglycemia results in a lowering of the oxygen utilization of the brain with consequent slowing of the potentials; in animals, this has been studied by Maddock, Hawkins, and Holmes (8), and in man, by Himwich (9) who have demonstrated that on insulin injection, the progression of clinical symptoms follows the increase in cerebral arterio-venous oxygen difference and this depression of cerebral metabolism is paralleled by a decrease in alpha frequency.

A similar correlation between brain metabolism and brain potentials has been found in cretins in whom the arterial-venous oxygen differences were reduced by thyroid administration (Himwich (10)). Lindsley and Rubenstein (11) and Ross and Schwab (12) have demonstrated a direct relationship in man between the frequency of alpha waves and the total calories per hour.

Further evidence for this theory is provided by the experiments on animals of Gellhorn and Kessler (13). These authors demonstrated that the action of hypoglycemia on the brain potentials can be offset by the inhalation of pure oxygen (provided that the hypoglycemia is not so severe as to induce convulsions). Conversely, the effect of anoxia on brain potentials is greatly aggravated during insulin hypoglycemia.

SUMMARY

Blood sugar levels below 70 mgm., but insufficiently low to impair consciousness, may have the following effects on the occipital potentials of the normal electroencephalogram during quiet breathing:

Slowing of the dominant frequency.
Development of activity slower than alpha.

No change has been observed in the following characteristics:

Percentage time alpha,
Percentage time beta,
Percentage time intermediate.

High blood sugar levels, induced by the inges-

tion of glucose (*i.e.*, above 130 mgm. per 100 cc.), do not affect the electroencephalogram.

CONCLUSION

In the assessment of the electroencephalogram for normality, the subject should take some glucose (50 to 100 grams) by mouth, 30 minutes before the test, to ensure a blood sugar level above 70 mgm. per 100 cc. In this way, changes in the electroencephalogram due to low blood sugar will be avoided; raising the blood sugar above 130 mgm. per 100 cc. does not alter the electroencephalogram pattern from that at ordinary blood sugar levels.

For technical assistance in this work the authors are indebted to Miss Margaret Gray and Mrs. Frances Cooperstein.

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CHARACTERISTICS OF THE NORMAL ELECTROENCEPHALOGRAM.

III. THE EFFECT OF VARYING BLOOD SUGAR LEVELS ON THE OCCIPITAL CORTICAL POTENTIALS IN ADULTS DURING HYPERVENTILATION [1]

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Delta activity, *i.e.*, waves of a frequency slower than 5 per second, is not a normal finding in the electroencephalograms of adults when they are fully awake and breathing quietly (1). This type of slow activity is found in some pathological states, but there are certain physiological factors which will cause it to appear in the record of the normal adult. The purpose of the present study was to investigate the effect of hyperventilation upon the occipital cortical potentials in a series of 45 normal subjects (29 males, 16 females), at varying blood sugar levels. The technique for the following experiments has been fully described in a previous paper (2).

If the blood sugar is lowered in normal subjects, delta waves will appear in the third minute of hyperventilation in nearly all cases. An example of one such case (female, age 21) is shown in Figure 1. This figure demonstrates clearly that in this subject, the brain wave pattern is a typical alpha type, stable at fasting blood sugar levels, where it is, in fact, identical with the tracing obtained at the higher level after the ingestion of glucose. Only at the artificially low blood sugar level of 50 mgm. per 100 cc. is the brain wave abnormal with trains of high voltage delta waves. All the tracings in this illustration (Figure 1) were taken during the third minute of hyperventilation.

Great individual variation was found within the group of 45 normals as to the level at which delta waves could be made to appear by hyper-

[1] This study was aided by a grant from the Harrington Fund.

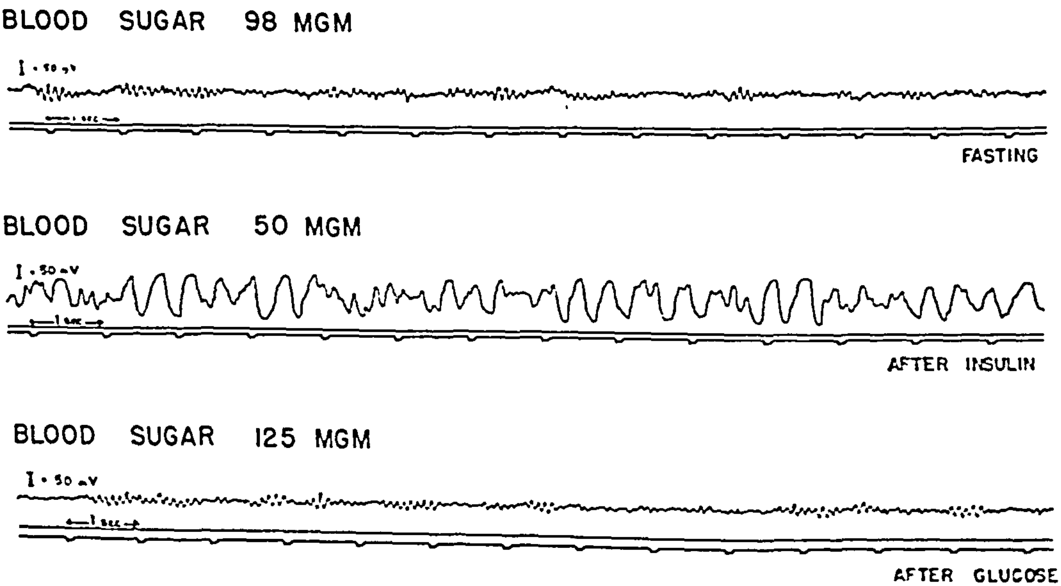

FIG. 1. DELTA ACTIVITY IN A NORMAL ADULT, PRODUCED BY HYPERVENTILATION AT LOW BLOOD SUGAR LEVELS

Three tracings from the same individual during hyperventilation at different blood sugar levels.

ventilation. In some (13 per cent of this series), they could not be elicited, even by lowering the blood sugar to 40 mgm. with insulin, whereas in a few (4 per cent), they were present at all levels, even after the ingestion of glucose.

The arbitrary criterion taken was the appearance of delta waves during at least 5 per cent of the third minute of hyperventilation (*i.e.*, for at least 3 seconds). At blood sugar levels higher than 70 mgm. per 100 cc., 31 subjects (69 per cent of the total series) were found to give at least this amount of delta activity.

Of these 31 subjects, 17 (38 per cent of the total series) still gave delta waves when the blood sugar level was raised to 100 mgm.; 5 subjects (11 per cent of the total) gave this amount of delta waves when the blood sugar level was as high as 130 mgm.

The clinical importance of these findings is their demonstration of the fact that, in the interpretation of brain wave records, delta waves in the third minute of hyperventilation cannot be regarded as an abnormal finding, since they occur in 38 per cent of normal adults at non-fasting blood sugar levels (100 mgm. or over). The importance of controlling the blood sugar level has also been stressed by Davis and Wallace (3, 4) as a result of their experiments on controlled hyperventilation of normals.

If the subject is given glucose by mouth before the test, or is examined within one-half hour of a meal in order to ensure a blood sugar of over 130 mgm., the number of normals giving delta waves falls to 11 per cent, but is still appreciable.

The records were then examined to determine whether the delta activity found in the second minute of overbreathing would be a better differentiating criterion. Seventeen (38 per cent) gave delta waves in the second minute for at least 5 per cent of the time when the blood sugar was kept at or above 70 mgm. Of these 17 subjects, 3 (7 per cent of the total series) still gave this amount of delta waves in the second minute when the blood sugar was raised to above 100 mgm. *No one* gave delta waves in the second minute when the blood sugar was kept at or above 130 mgm.

The same criterion, that is, regarding anything less than 5 per cent delta as negligible, was used in compiling the above figures; they are therefore strictly comparable with the figures for the third minute of overbreathing, and suggest that the differentiating threshold for delta activity should be the second minute of hyperventilation at blood sugar levels of 130 mgm. or over. Delta waves appearing for 5 per cent of the time in these circumstances may be regarded as an abnormal finding.

The percentage time delta in the third minute of hyperventilation at various blood sugar levels

This report has so far dealt with the number of individuals giving delta activity under certain experimental conditions. The next point to be examined is the amount of delta activity induced by the various factors, *i.e.*, the percentage time delta.

One hundred and sixty-one observations on 45 normal subjects were made at different blood sugar levels, and the mean percentage time delta

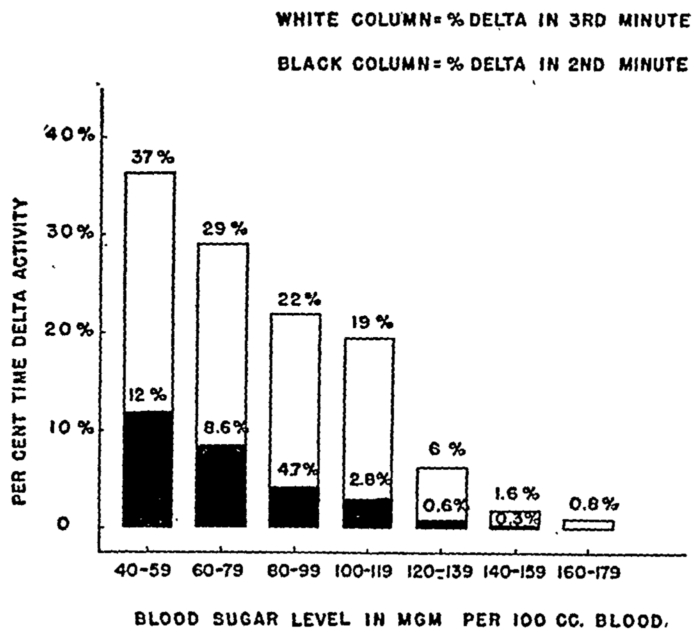

FIG. 2. PERCENTAGE TIME DELTA ACTIVITY ON HYPERVENTILATION

The above chart shows the percentage time delta activity in the second minute of hyperventilation (black columns), and in the third minute (white columns), in 45 normal adults. Where more than one observation at the same blood sugar level was made for one individual, the maximum amount of delta at this level is the one recorded. The number at the top of each column is the arithmetical mean for the group. The number of observations from which each column was computed were, reading from left to right: 11, 28, 39, 24, 31, 23, and 5.

in the third minute of hyperventilation was calculated at the various levels. The results are seen in the white columns of Figure 2. These are to be compared with the figures obtained on the same persons in the second minute of overbreathing, which are shown in the blackened portion of the columns in Figure 2. It is clear from this figure that there is a negligible amount of delta activity in the second minute of hyperventilation at non-fasting blood sugar levels.

Dominant frequency and tendency to delta activity on hyperventilation

The dominant frequency of the majority of waves in the occipital recordings was determined in this series by the distribution curve method, described in a previous paper (1). It was thought that possibly the slower the original rhythm, the more delta activity could be expected, but no significant difference in the amount of delta activity in the third minute of hyperventilation was found between the group with dominant frequencies slower than 10.5 and those with 10.5 cycles and faster. The figures are:

25 normals with dominant frequencies slower than 10.5 mean percentage time delta = 44.9 ± 27.8

19 normals with dominant frequencies of 10.5 and faster mean percentage time delta = 36.4 ± 19.7

The standard deviations of these means are so large that the difference between the 2 means is not significant. Thus, in this series, the lack of stability of the electroencephalogram to lowered blood sugar and increased ventilation shows no statistically significant relationship with the basic rhythm of the subject.

Depth of hyperventilation and its effect on delta activity

In addition to blood sugar level, there is another factor at work in determining the amount of delta activity which will occur, and this is the depth of hyperventilation. If the blood sugar level is kept constant, the amount of delta activity appearing in the third minute of hyperventilation will be significantly greater if the average respiratory volume during each of the 3 minutes of overbreathing is over 600 cc. per kgm. body weight, than if it is below this figure. Taking, as an example, the blood sugar range 80 to 99 mgm., there were 31 readings on subjects who breathed under 600 cc. per kilogram body weight in each of the 3 minutes of hyperventilation, and 18 readings on subjects who breathed over this amount. The mean percentage time delta in the third minute of hyperventilation for each of the two groups is shown below:

Under 600 cc. per kgm. body weight per minute ....Mean percentage time delta 10 per cent

Over 600 cc. per kgm. body weight per minute ....Mean percentage time delta 20 per cent

These figures reveal that there is an average of twice as much delta activity with the deeper level of breathing when the blood sugar is kept constant.

In 13 subjects, more than one observation was obtained at a blood sugar level between 80 and 99 mgm., but at different depths of hyperventilation. The results are given in Table I as the percentage time delta in the third minute of hyperventilation. The figures show clearly the influence of the greater depth of breathing upon delta activity, for the percentage time delta in the third minute is greater when the mean ventilation for the 3 minutes is increased.

The effect of age on the stability of the electroencephalogram during hyperventilation

A study of age relations was made in this series, although the age range was a very narrow one (17 to 38). It revealed that the breakdown into delta activity in the third minute of hyperventilation occurred more readily, *i.e.*, at higher blood sugar levels, in the younger members of the group. Examining once again the different groups which gave delta at widely different blood sugar levels, the following mean age for each group was found: (The results are given in order of decreasing stability)

2 subjects gave no delta at any level mean age 30

TABLE I

Percentage time delta in the third minute of hyperventilation at different depths of breathing, with the blood sugar kept constant

Mean depth of ventilation in the 3 minutes of hyperventilation (per kgm. body weight)

Subject	Under 500 cc.	500 cc.	Between 500 and 600	600 cc.	Between 600 and 700	700 cc. and over
			per cent			
5				18	41	
10			16	30		
13				2	13	
15			6	11		
18	49	61				
26			8	4		
27					10	35
28			10	24		
29			0	13		
35				73	84	
40			0			22
43	34	10			40	
44	6		31			

14 subjects gave delta only when the blood sugar was below 70 mgm. — mean age 25

17 subjects still gave delta when the blood sugar was above 100 mgm. — mean age 22

These groups are too small, and the age range too narrow for a statistical presentation of the age effect, but it would appear that the stability of the cortical potentials to hyperventilation increases with increasing age.

DISCUSSION

The appearance of high voltage slow waves as a result of voluntary hyperventilation is well known. This has been shown by Lennox and Gibbs (5, 6) to be due to increase in the carbon dioxide tension of the arterial blood reaching the brain, and not to anoxia, secondary to vasoconstriction. Berger (7) showed that slow waves can also be elicited by anoxia induced by rebreathing respired air from which the carbon dioxide has been removed; this has been confirmed by Gibbs and Davis (8) in human subjects breathing pure nitrogen, but the anoxemia has to be severe before any significant slowing takes place (Gibbs (9)). Himwich (10) has demonstrated an inverse relationship between oxygen utilization by the brain and delta frequency.

In the present paper, a study has been made of the interplay of the two factors: the influence of decreased carbon dioxide tension of the blood produced by varying depths of ventilation, and the anoxic effect on the cortex of lowering the sugar content of the blood.

SUMMARY

(*1*) Delta activity in the third minute of hyperventilation is not diagnostic of abnormality, since it occurs in 38 per cent of normals at non-fasting blood sugar levels. If the blood sugar level be raised above 130 mgm., 11 per cent of normals still give delta activity in the third minute.

(*2*) A better differentiation is given by the second minute of hyperventilation, since only 7 per cent of normals give delta activity at non-fasting blood sugar levels, and none above 130 mgm.

(*3*) The percentage time delta in normals varies inversely with the blood sugar level, in both the second and third minutes of hyperventilation.

(*4*) The tendency of an individual to produce delta activity on hyperventilation is not related to the basic dominant frequency of his resting record.

(*5*) When the blood sugar level is kept constant, the deciding factor in determining the amount of delta activity induced by hyperventilation is the depth of breathing during the 3-minute period.

(*6*) There is some evidence that at lower age levels in the adult, the electroencephalogram is

less stable to change in blood sugar and ventilation level.

For technical assistance in this work, the authors are indebted to Miss Margaret Gray and Mrs. Frances Cooperstein.

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THE EFFECTS OF PLEURAL EFFUSION ON RESPIRATION AND CIRCULATION IN MAN

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INTRODUCTION

Pleural effusion commonly complicates many diseases and its manifestations may alter or overshadow those of the underlying disorder. The severity of the respiratory symptoms associated with pleural fluid varies markedly from patient to patient and depends to some extent on the nature of the disease causing the effusion. The present study was made in an effort to gain a better understanding of the mechanisms responsible for the occurrence of and variations in respiratory manifestations in patients with pleural effusions. The effects of pleural effusion were estimated by comparing respiratory and circulatory dynamics before and after thoracentesis. The respiratory dynamics could be studied only in patients who were slightly or moderately dyspneic; accordingly, the findings of this study may not completely explain the symptoms occurring in patients with extreme degrees of discomfort.

MATERIAL AND METHODS

Eight patients, ranging in age from 22 to 72 years, were studied; 3 were males (Patients 1, 2, and 5). Patients 5, 6, 7, and 8 had congestive failure due to coronary artery sclerosis, associated in Patient 6 with thyrotoxicosis. Patient 4 had a pulmonary neoplasm. Patient 3 had a tuberculous effusion with no demonstrable parenchymal lesion. No definite diagnosis was made in Patients 1 and 2, although congestive failure, neoplasm, tuberculosis, and pneumonia were all ruled out; Patient 2 had a moderate degree of senile emphysema.

All studies were made with the patients sitting in bed with the trunk at an angle of 45° to 80° with the horizontal; approximately the same angle was used in all studies on a given subject. Measurements of the cardiac output were made with the patients in a basal state, 15 hours after the last meal. All other studies were made at least 2 hours after the patient's last meal and after he had rested in bed for at least forty minutes. The method of Starr and Gamble (1) was used for estimating the cardiac output, oxygen consumption, and respiratory tidal, and minute volumes being measured simultaneously. The functional residual air was determined by Christie's method (2), slightly modified (3), oxygen consumption and respiratory tidal, and minute volumes being measured simultaneously; reserve and complemental air volumes were measured separately. The venous pressure was measured by the direct method of Moritz and von Tabora (4), the circulation time by the decholin method (5), the arterial pressure by the auscultatory method using a standard cuff, and the arterial blood gas content by the method of Van Slyke and Neill (6), using blood obtained from the femoral artery.

OBSERVATIONS

The *functional residual* (*subtidal*) *air* was increased 18 hours after thoracentesis in 5 of the 6 patients studied, the increases ranging between 120 and 660 cc., or 5 and 31 per cent; the sixth patient showed an insignificant decrease of 35 cc., or 2 per cent. The average increase for the whole group was 301 cc., or 14 per cent. In the 2 patients studied up to 5 weeks after thoracentesis, further increases in functional residual air occurred, so that the final values were 805 and 1010 cc., or 38 and 40 per cent greater than the initial values (Table I).

The *residual air* increased in 3 patients, 18 hours after thoracentesis, by 245 to 450 cc., or 17 to 20 per cent. There was no change in one and insignificant decreases of 135 and 150 cc., or 8 and 10 per cent, in 2 patients. The average change for the whole group was an increase of 125 cc., or 6 per cent. There was no further increase in residual air in the 2 patients studied up to 5 weeks later.

The *reserve* (*supplemental*) *air* was greater after thoracentesis in every instance, the increase ranging between 100 and 320 cc., or 17 and 267 per cent of the initial value. The average increase was 176 cc., or 95 per cent. In the 2 patients studied up to 5 weeks later, the reserve air was further increased, so that the final values were 710 and 480 cc., or 592 and 400 per cent greater than the initial values.

TABLE I

Lung volumes and pulmonary dynamics before and after thoracentesis

Case	Functional residual air	Residual air	Reserve air	Comple-mental air	Vital capacity	Total capacity	Respira-tory rate	Tidal air	Respira-tory volume	Remarks
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per minute*	*cc.*	*liters per minute*	
1	2135	2015	120	1190	1310	3325	24	505	12.1	Before taps
	2795	2355	440	990	1430	3785	25	475	11.9	After taps—3400 cc.
	3060	2310	750	1670	2420	4730	19	475	9.0	Two weeks after taps
	3140	2310	830	1870	2700	5010	20	435	8.7	Five weeks after taps
2	2500	2380	120	870	990	3370	26	435	11.3	Before tap
	3070	2830	240	850	1090	3920	30	475	14.3	After tap—1100 cc.
	3510	2910	600	1860	2460	5370	23	495	11.4	Four weeks after tap
3	2200	1545	655	1210	1865	3410	23	360	8.3	Before tap
	2325	1395	930	1190	2120	3515	20	435	8.7	After tap—1200 cc.
4	1725	1645	80	1270	1350	2995	20	515	10.3	Before tap
	1690	1510	180	1350	1530	3040	16	560	9.0	After tap—1200 cc.
5	2555	1860	695	1530	2225	4085	21	360	7.5	Before tap
	2675	1860	815	1745	2560	4420	18	370	6.6	After tap—1000 cc.
6	1835	1240	595	1505	2100	3340	34	335	11.4	Before tap
	2200	1485	715	1530	2245	3730	25	435	10.9	After tap—700 cc.

The *complemental air* showed no constant or significant changes 18 hours after thoracentesis. There were increases of 25 to 215 cc. in 3 subjects and decreases of 20 to 200 cc. in the other 3; the range of percentage change was from plus 14 per cent to minus 17 per cent of the initial values. The average change was an increase of 13 cc., or one per cent. In the 2 patients studied up to 5 weeks later, the complemental air increased, so that the final values were 680 and 990 cc., or 57 and 114 per cent greater than the initial values.

The *vital capacity* increased somewhat, 18 hours after tapping. The increases ranged between 100 and 335 cc., or 7 and 15 per cent of the initial value; the average change was 189 cc., or 11 per cent. The increase after thoracentesis in each case was remarkably small compared to the volume of fluid removed. In the 2 patients studied up to 5 weeks later, further increases occurred, so that the final volumes were 1390 and 1470 cc., or 106 and 148 per cent greater than the initial values (Figure 1).

The *total capacity* increased somewhat, 18 hours after tapping. The increases ranged between 45 and 550 cc., or 2 and 16 per cent; the average increase was 314 cc., or 8 per cent. The change consequent to thoracentesis was small compared to the volume of fluid removed. In the 2 patients studied up to 5 weeks later, further increases occurred, so that the final volumes were 1685 and 2000 cc., or 51 and 59 per cent greater than the initial values.

TABLE II

Venous pressure and circulation time, before and after thoracentesis

Case	Venous pressure	Circulation time	Remarks
	cm. H_2O	*seconds*	
1	9.2	14.6	Before tap
	4.1	15.0	After taps totaling 3400 cc.
	3.7	16.1	Two weeks after taps
	4.0	16.5	Five weeks after taps
2	10.0	16.0	Before tap
	10.5	16.5	After tap—1100 cc.
	0.0	19.6	Four weeks later
3	7.9	12.9	Before tap
	4.8	13.6	After tap—1200 cc.
4	9.9	15.4	Before tap
	7.6	13.7	After tap—1200 cc.
5	9.5	26.9	Before tap
	6.4	25.7	After tap—1000 cc.
6	14.6	14.0	Before tap
	9.1	15.0	After tap—700 cc.
7	12.8	24.6	Before tap
	7.4	23.0	After tap—1300 cc.
8	16.5	41.4	Before tap
	11.1	35.0	After tap—2000 cc.

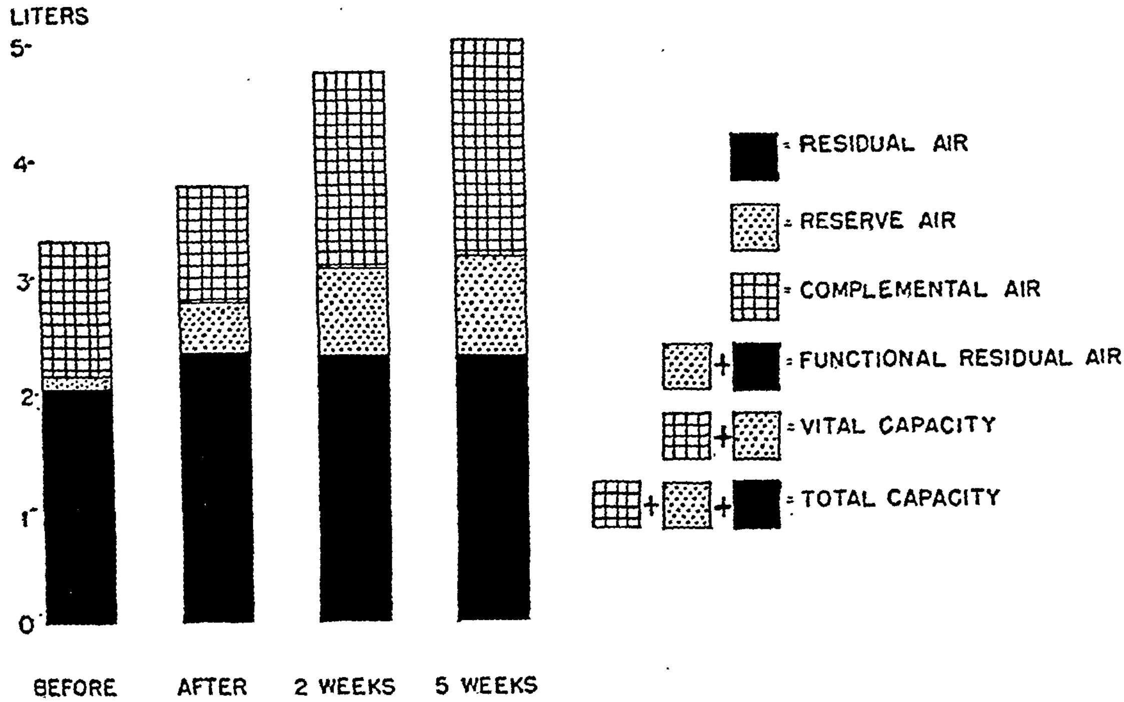

FIG. 1. SUBDIVISIONS OF THE LUNG VOLUME IN PATIENT 1 BEFORE AND AFTER THORACENTESIS

The *respiratory dynamics* showed no consistent changes, 18 hours after thoracentesis. The respiratory rate, high before tapping in all patients, fell in only 4. The average tidal air volume was slightly to moderately increased after thoracentesis in 5 of the 6 patients. The respiratory minute volume showed no significant change.

TABLE III
Cardiac output before and after thoracentesis

Case	Cardiac output	Cardiac output	Arterio-venous oxygen difference	Remarks
	L. per minute	*L. per 100 cc. O_2 consumed*	*volumes per cent*	
3	3.83	1.79	5.58	Before tap
	3.96	1.92	5.20	After tap—1200 cc.
5	2.66	1.08	9.24	Before tap
	2.70	1.05	9.52	After tap—1000 cc.

TABLE IV
Arterial blood gases before and after thoracentesis

Case	Oxygen saturation	CO_2 content	Remarks
	per cent	*volumes per cent*	
5	93.9	47.02	Before tap
	93.1	48.90	After tap—1000 cc.
7	95.7	42.55	Before tap
	96.9	42.43	After tap—1300 cc.
8	88.1	45.03	Before tap
	92.2	46.22	After tap—2000 cc.

The *venous pressure*, although within normal limits before thoracentesis in 5 of the 8 patients studied, fell immediately in all but one; the decreases ranged between 2.3 and 5.5 cm. of water. In the eighth patient, a subsequent decrease of 10 cm. occurred (Table II).

The *circulation time* and *arterial blood pressure* showed no consistent changes immediately after thoracentesis.

The *pulse rate* showed no significant change after thoracentesis; it varied from time to time in some of the patients, apparently in relation to changes in the degree of fever (Table III).

The *cardiac output* was studied in 2 patients, one (Patient 5) with congestive failure and the other (Patient 3) with no heart disease. The cardiac output before thoracentesis was low in the former and normal in the latter; there was no change 18 hours after tapping (Table IV).

The *arterial blood gases* were not remarkable before thoracentesis and were not influenced by removal of the pleural fluid in 2 of the 3 patients

studied. In the third, the arterial oxygen saturation was 88.1 per cent before, and 92.2 per cent one hour after the chest tap.

DISCUSSION

Although many acceptable data on the vital capacity in patients with pleural effusion exist, reported observations (7 to 9) on the other subdivisions of the lung volume are fragmentary and were made by methods no longer considered reliable. In the present study, no analysis of the findings before thoracentesis was made, since most of the patients had some parenchymal disease in addition to the effusion. Significant changes were, however, found after thoracentesis in all the subdivisions of the lung volume except the residual and complemental airs; the changes in residual air may possibly be significant.

The functional residual air rose somewhat immediately after thoracentesis and therefore it is concluded that pleural effusion causes atelectasis, the collapsed lung re-expanding to some extent with removal of the thoracic fluid. Complete re-expansion, as measured by the functional residual air, did not, however, occur for 3 or 4 weeks. Accordingly, overcoming of the atelectasis must be regarded as only of contributory importance in the immediate relief of dyspnea which may occur with thoracentesis. Since the removal of large amounts of fluid from the chest is followed by an immediate increase in functional residual air of only a few hundred cubic centimeters, it is clear that elevation of the diaphragm must occur during thoracentesis. The diaphragm, pushed down and flattened by pleural effusion, resumes its normal arched contour as fluid is withdrawn. In its depressed, flattened state, the diaphragm is in a position which permits only limited excursion and makes for inefficient respiration; after resumption of its normal arch, diaphragmatic respiratory excursion is greatly increased and respiration becomes more efficient. This is in harmony with the clinical observation that patients with dyspnea associated with pleural effusion show active use of the accessory muscles of respiration which is abated by thoracentesis.

Of the two components of the functional residual air, *i.e.*, the residual and reserve airs, the former is only slightly affected by pleural fluid, whereas the latter is markedly decreased. Other authors have pointed out that diminution in the volume of the reserve air in such diseases as emphysema (10) and congestive heart failure (11) is associated with decreased negativity of the intrapleural pressure; as the reserve air approaches zero, the intrapleural pressure approaches atmospheric. Accordingly, it is to be concluded from the data of the present study that the intrapleural pressure in patients with fluid in the pleural spaces is less negative than normal. Indeed, measurements of the intrapleural pressure in such patients have been recorded by Clark (12) and by Shattuck and Welles (13) and demonstrate the loss of all or most of the normal negative pressure. Following thoracentesis, the reserve air increases markedly and the intrapleural pressure has been shown to become more negative (12, 13). Decreased negativity of the intrapleural pressure, consequent to any cause, impairs the efficiency of respiration and also influences cardiovascular dynamics in a manner which will be discussed below.

The complemental air, a measure of the expansibility of the lungs, is markedly diminished by pleural fluid. This fluid acts to decrease pulmonary expansibility in two ways: (*1*) by occupying space within the thorax and (*2*) by causing atelectasis, the atelectatic lung being less expansible than the normal. Although the complemental air in the patients of the present study has never decreased to the volume of the resting tidal air, it was in some instances sufficiently small to prevent the normal increase in tidal air during exercise. Indeed, Knipping (14) found a decrease in maximal respiration during exertion in patients with pleural effusion. The decreased complemental air of pleural effusion therefore makes for anoxia during exertion and consequently contributes to dyspnea. Decreased arterial oxygen saturation was found in only one patient here studied at rest, but would probably occur in all during severe exertion. Ihaya (15) found lowered arterial blood oxygen saturation in most of his patients at rest. Impaired expansibility of the lungs also favors dyspnea by requiring that the patient expend more effort in attaining a given tidal air volume; in the present study, the tidal air volume was decreased

before the removal of fluid. The complemental air is affected little by thoracentesis, attaining its normal volume only with complete re-expansion of the atelectatic lung in the weeks following removal of the fluid.

Early observers of the vital capacity noted (16, 17) that the vital capacity is low in patients with pleural effusion, but is only slightly increased immediately after thoracentesis; the findings of the present study are in harmony with these earlier observations. The vital capacity is the sum of the reserve and complemental airs; the latter is much larger than the former, so that its lack of change overshadows the marked changes in reserve air after pleural fluid is removed. The vital capacity returns to normal with the complemental air some weeks after chest tap. It is clear that study of the vital capacity in patients with pleural effusion affords no accurate information on the state of pulmonary function.

The total lung volume is decreased by pleural effusion and increases only slightly after thoracentesis, reaching normal levels several weeks later.

The above-discussed changes in intrapleural pressure in patients with pleural effusion impair venous return, as other authors pointed out (18, 19). In the present study, although the venous pressure was not elevated above normal in the patients without congestive failure, it fell following thoracentesis; this finding corroborates earlier observations by others (12, 26). The finding of Hitzig (20) that the venous pressure is within normal limits in patients with pleural effusion is not to be considered contradictory, since he did not study his patients before and after tapping. The fall in venous pressure which occurs after thoracentesis is of particular interest in that it may explain the occurrence of diuresis in some cardiac patients following this procedure. Impairment of venous return may result in decreased cardiac output. However, Ringer and Altschule (21), using the Henderson-Haggard ethyl iodide method, obtained values normal for that method in patients with pleural effusion, and Ihaya (15), who used the acetylene method, also found no striking deviation from normal. In the 2 patients studied here by the Starr and Gamble ethyl iodide method, a normal cardiac output was found in the one with no heart disease and decreased values in the patient with congestive heart failure, with no change in the output of the heart in either after tapping. Busacchi (22), using the acetylene method, found markedly decreased cardiac outputs in most of his patients with pleural effusion, with return to normal following clearing of the fluid. His observations, however, are not entirely applicable to the present discussion, since at least some of his patients had adhesive mediastino-pericarditis. Moreover, his data on normal subjects are so variable as to suggest some error in procedure. It is therefore concluded that pleural effusion does not affect the cardiac output in patients at rest. This is in harmony with the observations on decholin circulation time reported here and on saccharine and ether times recorded earlier by Hitzig (20). Although some obstruction to the return of blood from the periphery exists because of decreased negativity of intrapleural pressure, it appears that enough pressure is built up in the veins so that flow is not decreased and therefore cardiac output remains unchanged in these patients at rest. It is probable, however, that the increase in cardiac output in exercise in patients with hydrothorax would be less than normal. Evidences of impaired cardiac function, consequent to extreme degrees of pressure on the heart or of mediastinal displacement, were not observed in the present study, but this does not rule out their occurrence in patients with larger amounts of fluid in the pleural spaces.

It is clear from all of the above discussion that pleural effusion acts in many ways to impair respiratory and cardiac function. Nevertheless, many patients with large effusions exhibit little or no discomfort, at least while at rest. The results of the present study show that the changes in pulmonary physiology caused by hydrothorax are similar to those consequent to emphysema (11, 23), diffuse pulmonary fibrosis (24), and chronic congestive failure (10, 25). Accordingly, patients with diffuse pulmonary disease, *i.e.*, emphysema, fibrosis, or congestion, are more likely to exhibit dyspnea and orthopnea when pleural fluid develops than other patients. The severity of these respiratory symptoms varies, not only with the volume of fluid in the

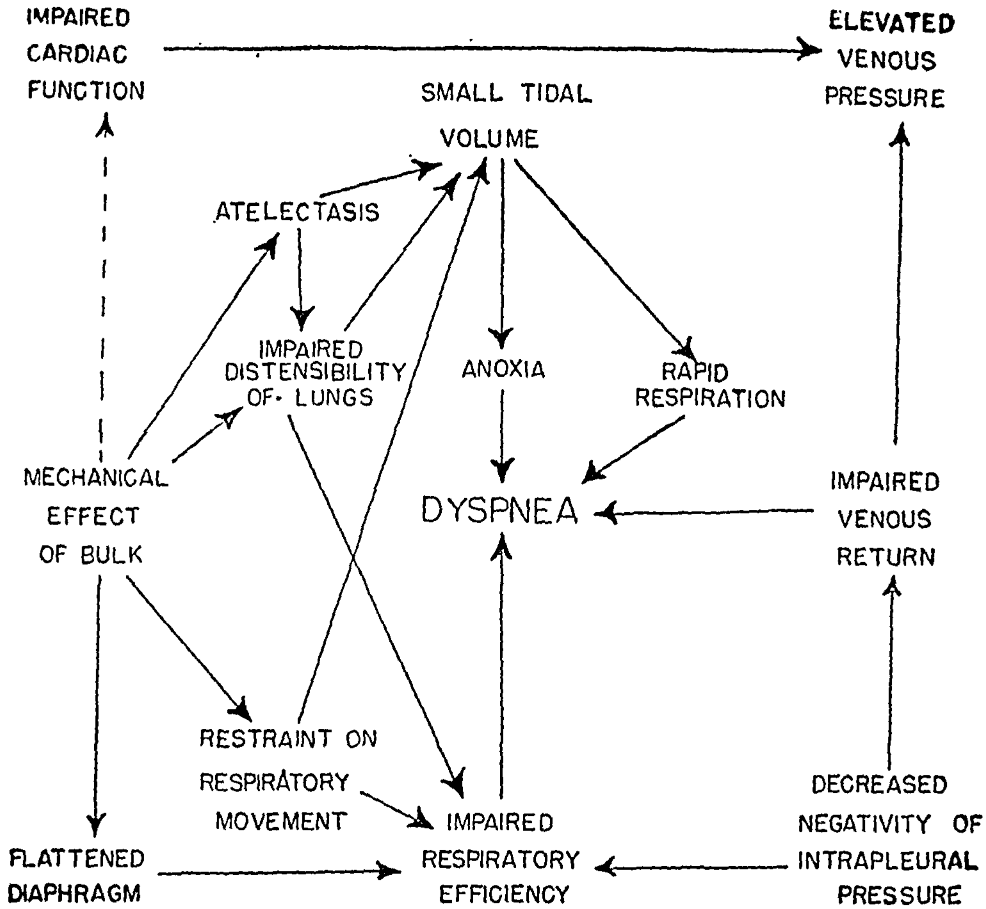

FIG. 2. SOME MECHANISMS FAVORING DYSPNEA IN PATIENTS WITH PLEURAL EFFUSION

pleural spaces, but also with the severity of the underlying pulmonary disease. Conversely, if a patient obtains marked relief from respiratory discomfort following a relatively small thoracentesis, it is likely that he also has some diffuse pulmonary lesion (Figure 2).

The effects of thoracentesis may be summarized as follows:

A. *Immediate*
1. Increased negativity of intrapleural pressure
   a. improved respiratory efficiency
   b. improved venous return
2. Removal of bulk of fluid
   a. removal of restraint on respiration
   b. restoration of diaphragmatic arch
3. Some re-expansion of collapsed lung

B. *Late*
1. Re-expansion of atelectasis
   a. increased respiratory space
   b. restoration of expansibility of lung

SUMMARY AND CONCLUSIONS

1. The effects of pleural effusion have been estimated in 8 patients by comparison of measurements of the lung volume and respiratory and cardiovascular dynamics before and after thoracentesis.

2. Atelectasis, decreased expansibility of the lungs, decreased negativity of intrapleural pressure, and shallow respiration are consequences of pleural effusion; anoxia may occur.

3. Increased peripheral venous pressure, a manifestation of impaired venous return consequent to changes in intrapleural pressure, is caused by pleural effusion. There are no changes in cardiac output or circulation time, at least at rest, as a consequence of pleural fluid.

4. It is concluded that pleural effusion impairs respiration and circulation in many ways, thereby favoring the occurrence of dyspnea and orthopnea; these symptoms will be most severe in patients who have extensive diffuse disease of the lungs in addition to the effusion.

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THE INFLUENCE OF THE COLLAPSIBILITY OF VEINS ON VENOUS PRESSURE, INCLUDING A NEW PROCEDURE FOR MEASURING TISSUE PRESSURE

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The study of the venous pressure by the direct method of Moritz and von Tabora (1) has been of great value in the diagnosis and physiological understanding of heart failure and a number of other conditions. Provided a certain technique in measurement, as outlined by Lyons, Kennedy, and Burwell (2), is observed, it is commonly assumed that the arm venous pressure is a function of the atrial pressure, since the vein is a tube connecting the antecubital space with the heart. It is our purpose to show that the peculiar nature of this tube, in that it is easily collapsible, places important restrictions on this interpretation.

The critical experiment in this regard is the classical phenomenon, studied by many observers, of the behavior of the venous pressure as the arm is raised (Fig. 7). When the pressure is referred to the heart level, the pressure is constant until the arm reaches a certain point, and then rises equally with further elevation. When the pressure is referred to the vein level, the pressure falls until this same point is reached, and then remains constant. Observation indicates that below this point, the vein is distended, and at and above this point, the vein is collapsed.

It is with the full interpretation of this phenomenon that this paper deals. It is our purpose to show that when the vein is distended, it does measure the central venous pressure, and that when it is collapsed, it is completely independent of central influences and measures only the tissue pressure about the vein at the point of measurement. This will be done by showing, first, that a collapsible tube behaves in this way; second, that an isolated segment of vein has the properties of a collapsible tube; and third, that the vein behaves *in vivo* just as it does *in vitro*.

MATERIALS AND METHODS

Static experiments. The static experiments were done on a rubber glove and on excised veins.

The distensibility curves of a rubber glove were obtained as follows. A glass tube was tied into the rubber glove, and bent in such a way that negative as well as positive pressures could be read, and a side tube was connected so that increments of fluid could be added. The rubber glove was stuffed into a 250 cc. graduate, mounted horizontally, and closed at the open end by a two-holed rubber stopper. A glass tube was bent in the shape of a U, and so connected to the graduate that the pressure in the graduate could be read by measuring the difference in height of the water columns in the U tube. A side arm was added for applying a desired pressure and readjusting the pressure after increments of water were added to the glove.

An internal mammary vein which was apparently normal was obtained 12 hours post-mortem. It was mounted in an analogous fashion to the rubber glove, but with refinements such that increments of volume change could be measured after adding fluid from a micro-burette, accurate to ±0.005 cc. The pressure changes were recorded in a capillary of such cross-sectional area that 0.01 cc. of fluid occupied 2.4 cm. of its length. Pressures were read to the nearest millimeter of water, and corrections were made for the volume change in the manometer. All connections were glass to glass, or by pressure tubing. The vein was mounted horizontally, and placed under very slight longitudinal tension by fitting in two small copper wires. This was necessary to allow saline to enter the vein and be distributed throughout its length; otherwise, its walls stuck together.

A grossly normal common iliac vein was obtained 8 hours post-mortem. It was mounted in a fashion similar to the internal mammary vein, except that the volume was read only to an accuracy of 0.1 cc. Like the rubber glove, it was enclosed in a graduate so as to permit the control and measurement of ambient pressure variations.

Dynamic experiments. The dynamic studies were done on a thin walled latex tube and a common iliac vein.

The model consisted of a latex tube, 5.36 cm. in circumference, with a wall, 0.030 cm. in thickness, mounted at about 30° to the horizontal. It was secured at each end around glass tubes of the same internal circumference. The ends of the glass tube were 34.8 cm. apart. Tap

water was admitted at the higher end at the rate of 243 cc. per minute by pressure tubing. The lower end was connected to rubber tubing, the outflow level of which could be varied with respect to the level of the latex tube.

A glass capillary was led into the center of the latex tube from one end, and connected to a water manometer. The latex tube, so arranged, was mounted inside a glass cylinder, closed at both ends by rubber stoppers, through which were passed the glass tubes on which the latex tube was mounted. The pressure in this air-filled cylinder was measured by the difference in height of the water in the two arms of a U-tube, one end of which was connected to the cylinder, and the other to the outside air. A syringe and needle arrangement permitted the maintenance of a desired pressure.

The model made possible the study of the pressure changes inside the latex tube at given volume flows of water, with different external pressures, with varying outflow levels, with the tube raised or lowered, and inspection of the visible changes in the shape of the latex tube under these conditions.

The analogous parts of the *in vivo* system are the vein to the latex tube, the cuff about the arm to the closed air space about the tube, the atrial level to an arbitrary reference point, and the atrial pressure to the distance of the outflow level above this arbitrary reference point. To avoid confusing terminology, the different parts of the model will be named after the analogous parts of the *in vivo* system. With reference to the arbitrary reference point ("atrial level"), the outflow level will be called the "atrial" pressure, the pressure in the tube, the "central venous pressure," and the level of the catheter tip in the tube, the "vein" level. With reference to the "vein" level, the pressure in the tube will be called the "local venous pressure."

If the pressure readings are considered to be in absolute units, so that the heights of the fluid columns in the manometers can be read as pressures, then the above definitions establish a relation between the pressure and the two reference points ("vein level" and "atrial level") as follows: $VL = CVP - LVP$, where VL is the vein level, CVP is the central venous pressure (height of fluid column above atrial level), and LVP is the local venous pressure (height of fluid column above vein level). Because of this relation, the observed values are plotted on triaxial coordinates, in order that the relation of each pressure reading to the two reference points can be most advantageously shown.

In vivo *studies.* The *in vivo* studies were done on a variety of subjects, in all stages of health and disease. None had obvious local disease of the veins.

The venous pressures were determined by the direct method of Moritz and von Tabora (1), as modified by Lyons, Kennedy, and Burwell (2), with the patients lying supine. A minor but convenient modification in their

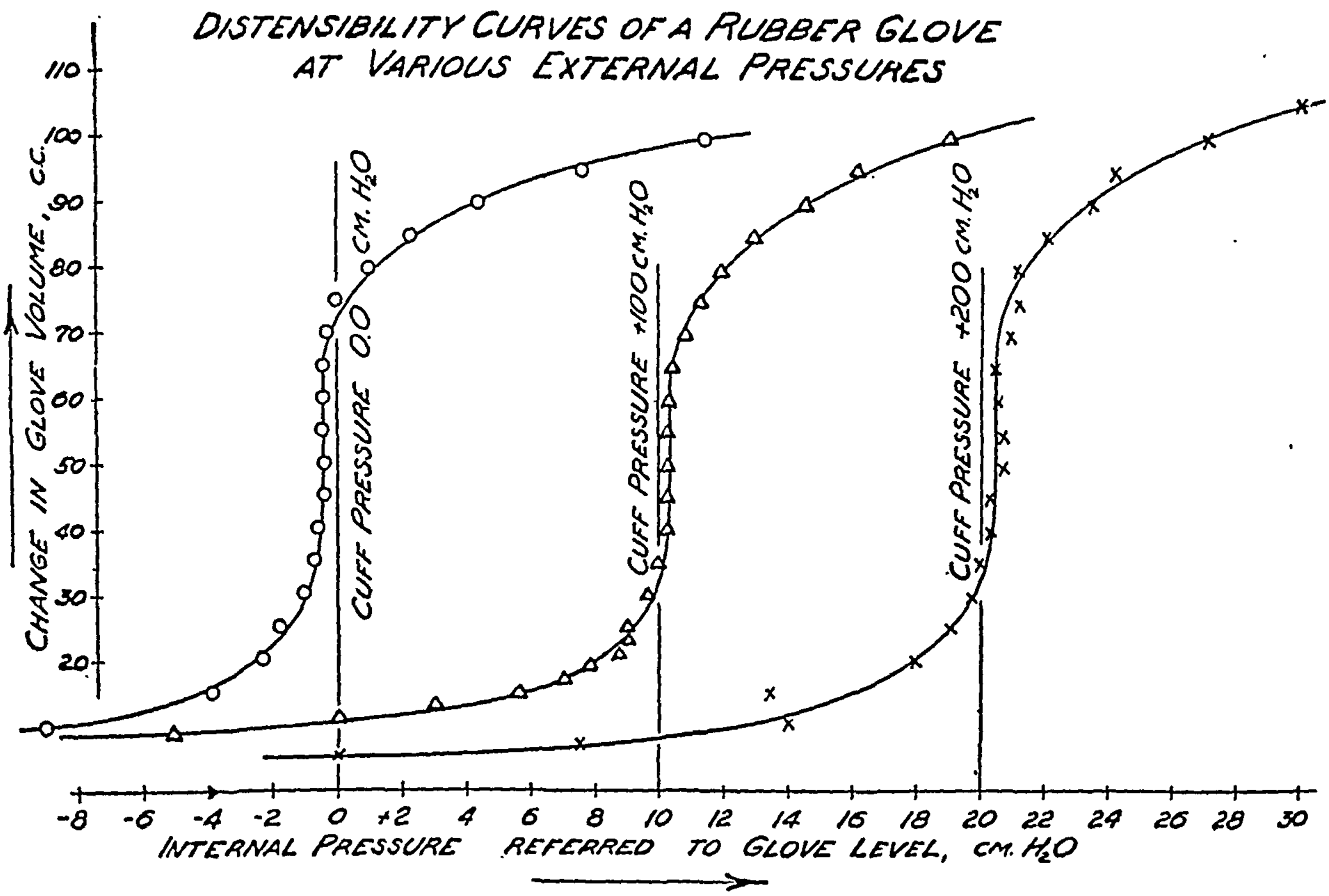

FIG. 1. DISTENSIBILITY CURVES OF A RUBBER GLOVE AT VARIOUS EXTERNAL PRESSURES

Considerable increments of fluid may be added to the glove without change in internal pressure, within certain limits of volume change of the glove. The internal pressure is equal to the external pressure during this phase.

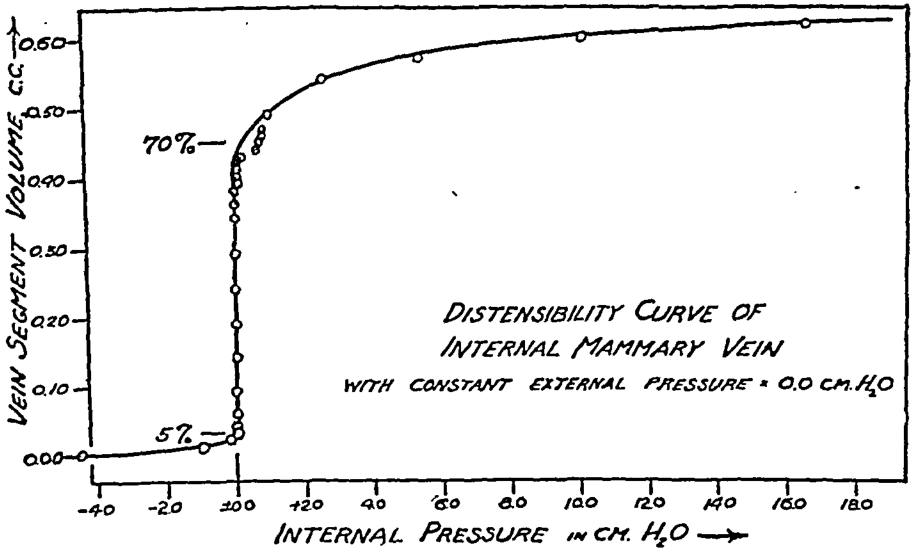

FIG. 2. DISTENSIBILITY CURVE OF AN INTERNAL MAMMARY VEIN SEGMENT
For a wide range of volume change, the internal pressure reflects the ambient pressure.

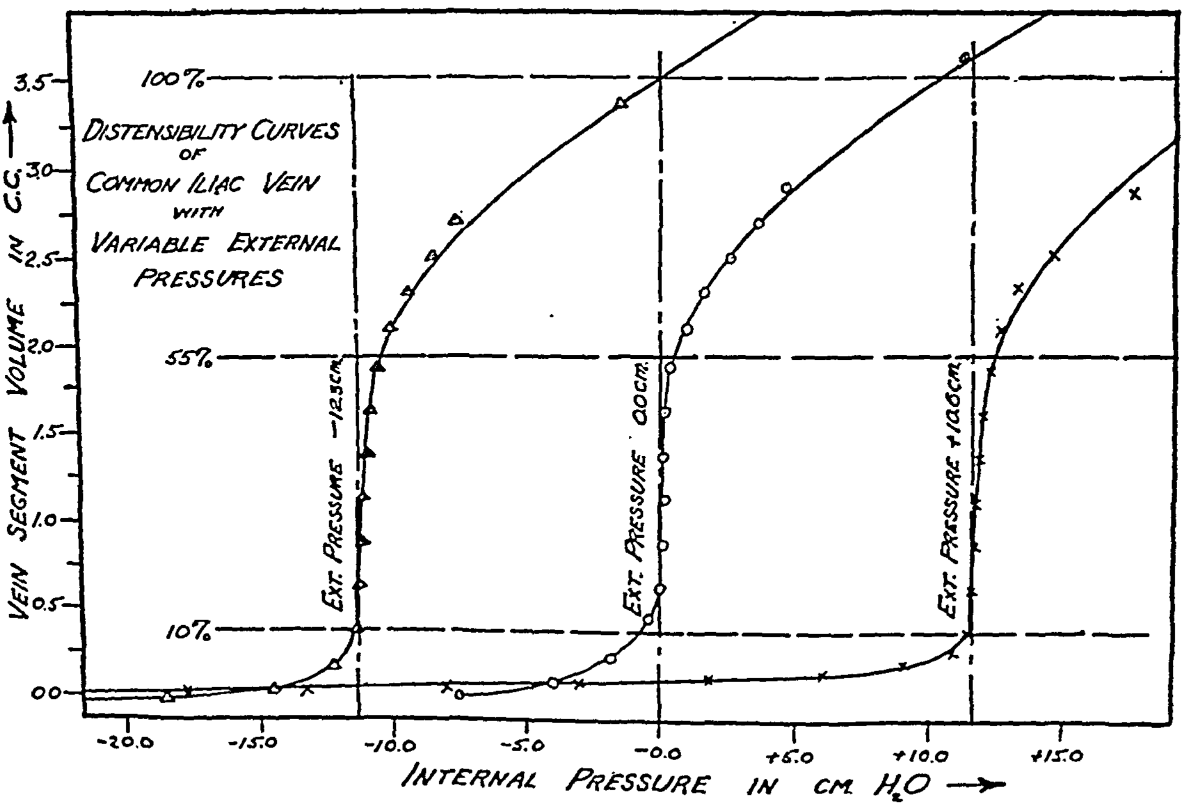

FIG. 3. DISTENSIBILITY CURVE OF A COMMON ILIAC VEIN SEGMENT
For a range less wide than in the smaller vein, the internal pressure reflects the ambient pressure.

procedure was the method of establishing the manometer level of the atrium. We measured the antero-posterior chest diameter from the angle of Louis to the spine of the fourth thoracic vertebra with obstetrical calipers, and then by means of a spirit level related the manometer to the angle of Louis so that the manometer zero (atrial level) was 10 cm. above the spine of the fourth thoracic vertebra. In some of the studies, a vein on the dorsum of the forearm was used instead of the antecubital vein. Normal saline was usually used without an anticoagulant. The pressure readings were corrected for capillarity when necessary. The vein levels were determined by feeling the tip of the needle in the vein, and leveling accordingly. Particularly when a cuff is in place over the needle tip, the error in leveling may be as great as 1 cm.

OBSERVATIONS

Static properties of collapsible tubes and veins. When increments of volume are added to a thin walled bladder, the pressure-volume relations follow a sigmoid curve, with a long vertical rise of volume change without measurable pressure change, representing a state of free distensibility [1] (Figure 1). If the total capacity of the bladder (or segment) is arbitrarily taken as the volume change effected by internal pressures from 10 cm. water below to 10 cm. above the ambient (surrounding) pressure, the range of free distensibility relative to total capacity is wide and becomes wider the thinner the bladder wall. In this range of free distensibility, the internal pressure is equal to the ambient pressure. Isolated vein segments behave in the same way and likewise, their range of free distensibility depends upon the properties of the vein wall (Figures 2 and 3), becoming less as the thickness of the wall increases.

Dynamic properties of collapsible tubes and veins. At volume flows just below those which induce bumping, the latex tube is found to have properties analogous to those of the bladder, studied under static conditions. The tube is collapsed when it is some distance above the outflow level, and distended when it is below the outflow level. When the tube is collapsed, it mirrors exactly the ambient pressure, as measured in a cuff about the tube; and it is quite independent of any level at which the outflow is set, provided the outflow level is not high enough

[1] The terms "free distensibility" and "free collapsibility" are used interchangeably throughout this paper, and are considered to denote the range of free volume change.

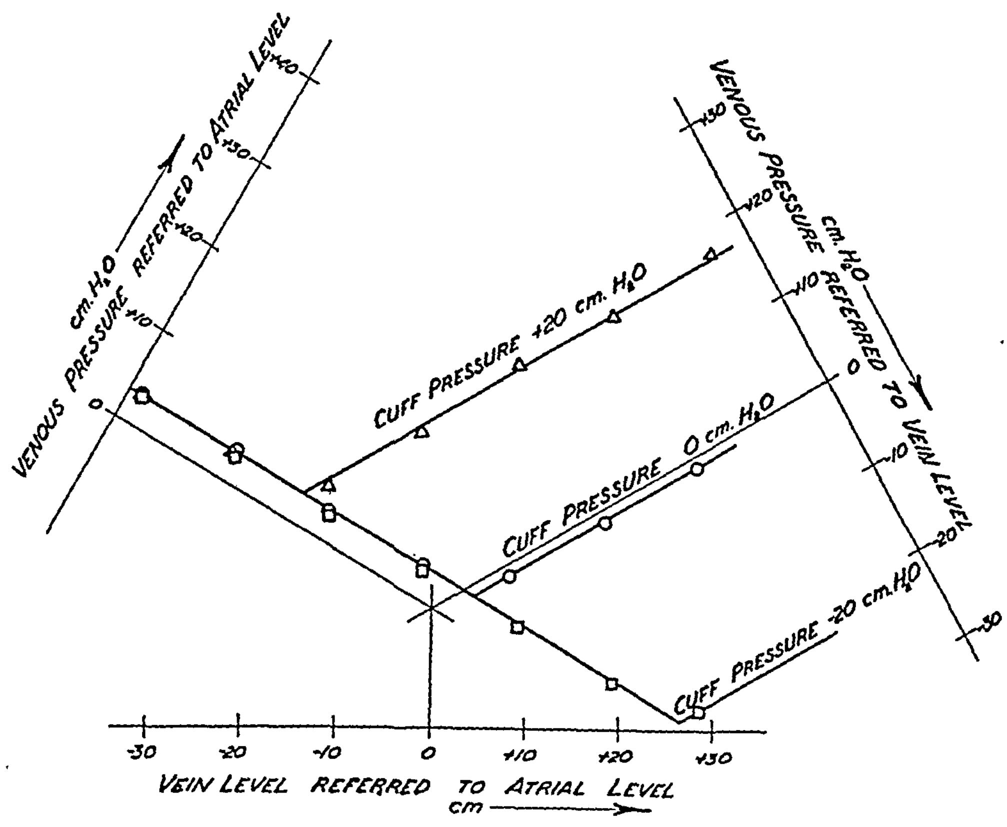

FIG 4. DYNAMIC MODEL EXPERIMENTS

For description of terms, see text under the description of the model. The pressure inside the tube, "venous pressure," is constant in reference to the "atrial level" when the tube is distended by the "atrial pressure." As soon as the tube is collapsed by increasing the external cuff (ambient) pressure, the internal pressure mirrors this pressure, and is independent of the "atrial pressure."

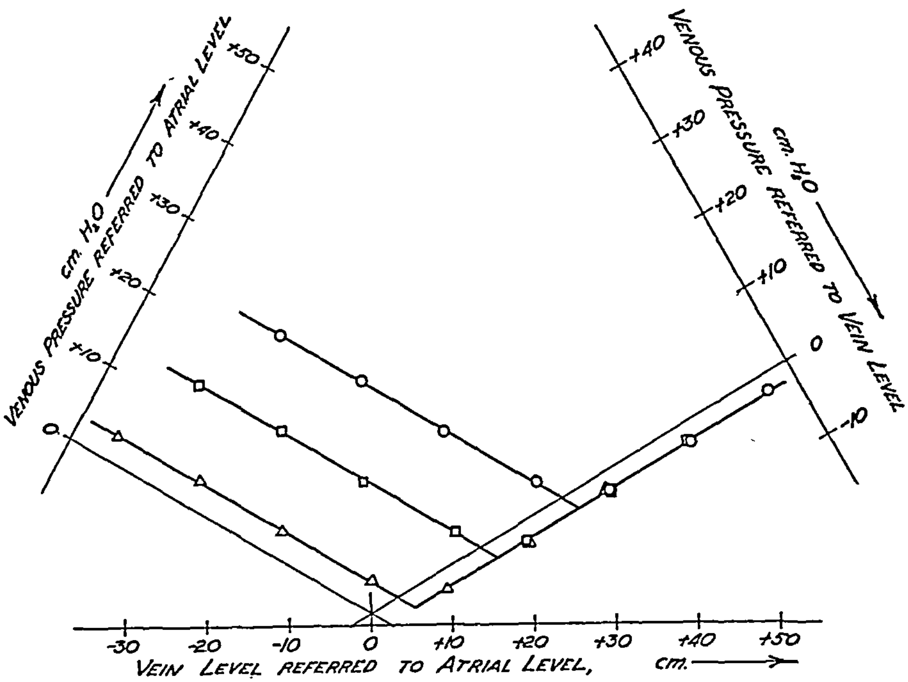

FIG. 5. DYNAMIC MODEL EXPERIMENTS

When the "atrial pressure" is changed by changing the outflow level, the pressure in the tube reflects the change in the "atrial pressure," remaining constant at the "atrial pressure" as long as the tube is distended, no matter what the "vein level" is. The internal pressure is independent of the "atrial pressure" as soon as the "vein" collapses when sufficiently elevated.

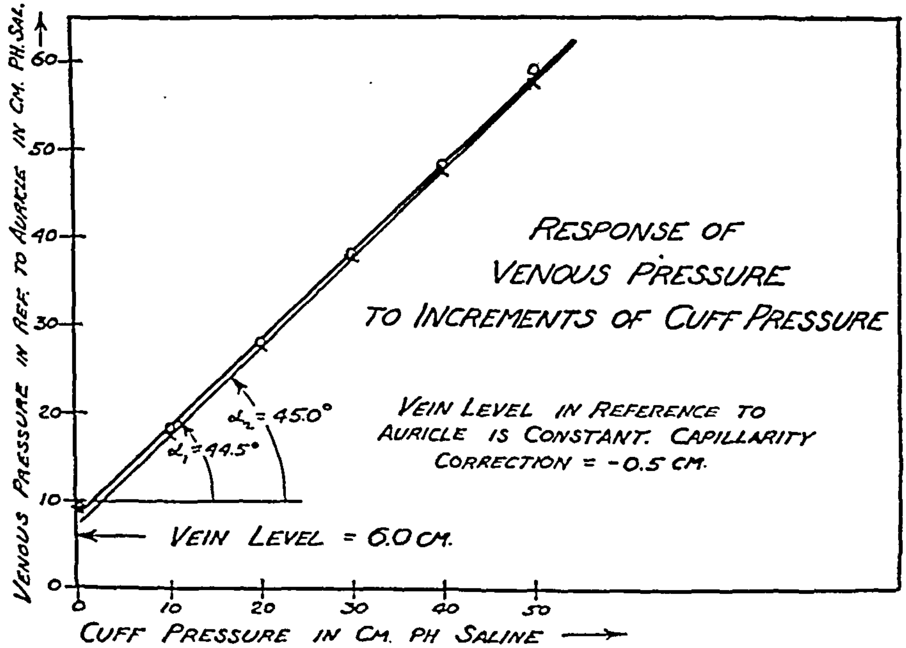

FIG. 6. COMPLETE TRANSMISSION OF CUFF PRESSURE TO A FOREARM VEIN, *in Vivo*

The cuff was placed about the forearm vein with the vein at the indicated level, and the cuff then inflated to 50 cm. of pressure. The venous pressure was recorded at that cuff pressure, and at successive 10 cm. decrements of cuff pressure. After the first run, denoted by circles, the cuff was slightly readjusted, and the second curve obtained. The applied cuff pressure was completely transmitted the second run.

to distend the tube and the volume flow is in the proper range. When the tube is distended, its internal pressure equals the level of the outflow tube and is quite independent of the ambient pressure changes (Figures 4 and 5).

The isolated vein behaves in quite the same way.

Veins in vivo. The procedures on arm veins *in vivo* are anlogous to the above experiments. When the vein is elevated to the point of collapse, and a cuff is placed around the arm and overlying the vein, increments of pressure added to the cuff are exactly transmitted to the lumen (Figure 6). When the vein is distended by holding it below the body level, increments of cuff pressure do not affect the lumen pressure at all, until the cuff pressure exceeds the lumen pressure.

A slight modification in the procedure is necessary to show conveniently the dependence of the pressure in the distended vein on more central influences. When the arm of any individual is raised from a position of maximal depression to one of maximal elevation, and the venous pressure is measured continuously, the venous pressure curves, plotted in reference to the atrial and vein levels, are characteristic (Figure 7). The use of individuals with different central pressures (referred atrial pressure) changes the magnitude of the pressure but not the form of the curve (Figure 8). The application of arbitrary cuff pressures over the vein does not affect the venous pressure as long as the vein is distended, but does affect the pressure when the vein is collapsed, either by elevation or by a sufficiently high cuff pressure (Figure 9). Individuals with different central pressures show constant differences as long as their veins are distended (Figure 8). The veins in this experiment behave *in vivo* just as the tube does *in vitro* as the level of the tube is raised.

The effect of tissue pressure. The constant positive hydrostatic pressure difference between the local venous pressure and the vein level,

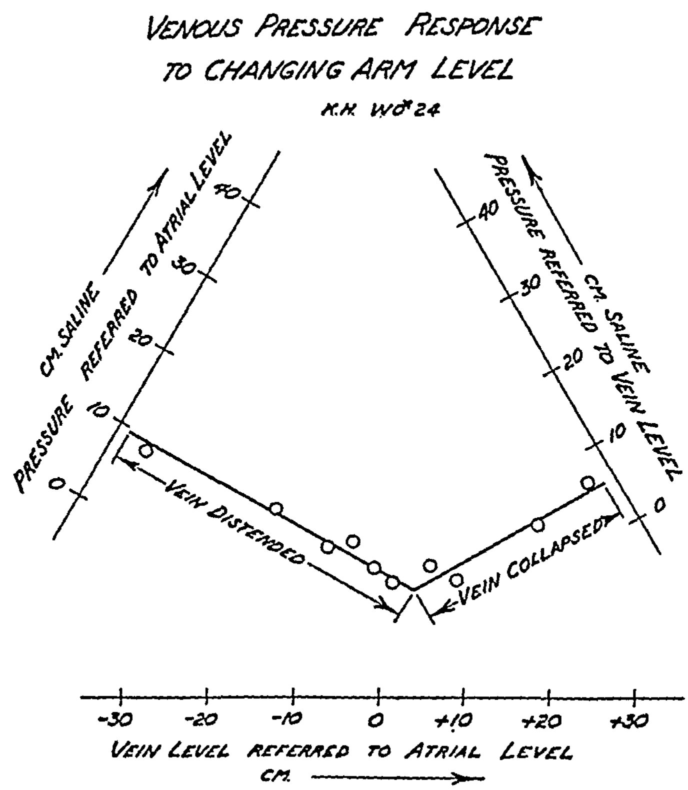

FIG. 7. VENOUS PRESSURE RESPONSE TO CHANGING ARM LEVEL

As the arm is raised from a dependent position, beginning with the arm vein 27 cm. below the atrial level, the vein remains distended and the pressure remains constant in reference to the atrial level, but falls in relation to the vein level, until a point 4 cm. above the atrium is reached. At this point, the vein collapses, and above it, the pressure in reference to the vein level remains constant while the pressure in reference to the atrial level appears to rise.

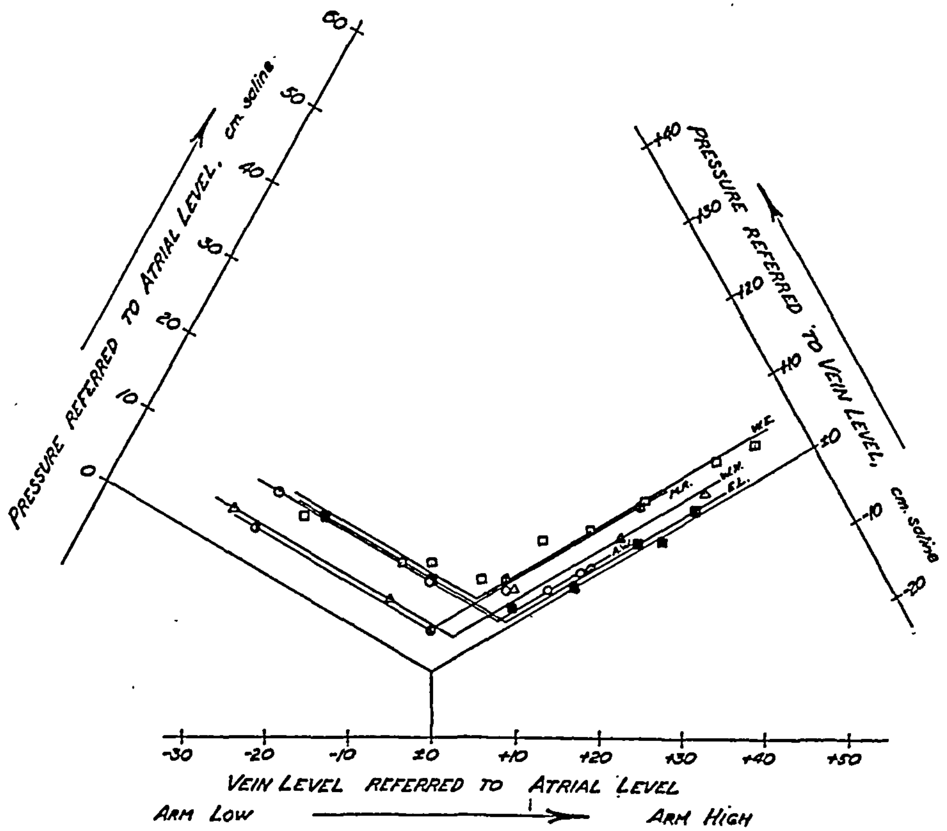

FIG. 8. PRESSURE RELATIONS IN DIFFERENT SUBJECTS

The pattern of the pressure changes is the same for all the individuals, but the pressure levels are characteristic for each individual.

when the vein is any distance above the point of collapse, is quite analogous to the local pressure in the collapsed rubber tube, which has been shown to be a function of the ambient external pressure. The fact that changes in cuff pressure are completely transmitted to the lumen of the vein (Figure 6) proves that under these conditions the vein is freely collapsible, since the vein wall does not resist this *complete* transmission of pressure. If the tissue pressure is defined as the hydrostatic pressure exerted by the tissue on any point in the tissue, the residual pressure in the vein must then be exactly equal to the tissue pressure about the vein.[2]

[2] If the local pressure in a collapsed vein is to be interpreted as being due solely to tissue pressure about the vein, measurable tonus in the vein wall must be absent, and the volume flow must be such that the vein is not collapsed beyond its range of free collapsibility; proof that both conditions obtain derives from the fact that under the conditions of the experiment the vein *is* freely collapsible. On the other hand, in the course of these studies we have been able to induce *measurable* spasm of large superficial arm veins and to relax this spasm at will (3), and we have observed certain collapsed veins (internal jugular) *in vivo* in which the local pressure was a function of external pressure (4), but not the sole function. It can be stated, first, that under ordinary circumstances the arm veins are free of measurable tonus and the flow through them is such that the veins cannot collapse beyond the free range of volume change, and hence the local pressure in the collapsed arm vein is a measure solely of tissue pressure; and second, that the presence of venous spasm can be detected by local observation and palpation because the spastic vein is small, round, and firm, in contrast to the flat, soft character of the limp collapsed vein.

In this connection, it is of interest to compare our observations on the tissue pressure, as measured in this way, with those of Burch and Sodeman, measured by determining the pressure at which fluid could be forced into the tissue (Table I). There is no significant difference between the means of the two groups. Considering that the individuals are different, the sites

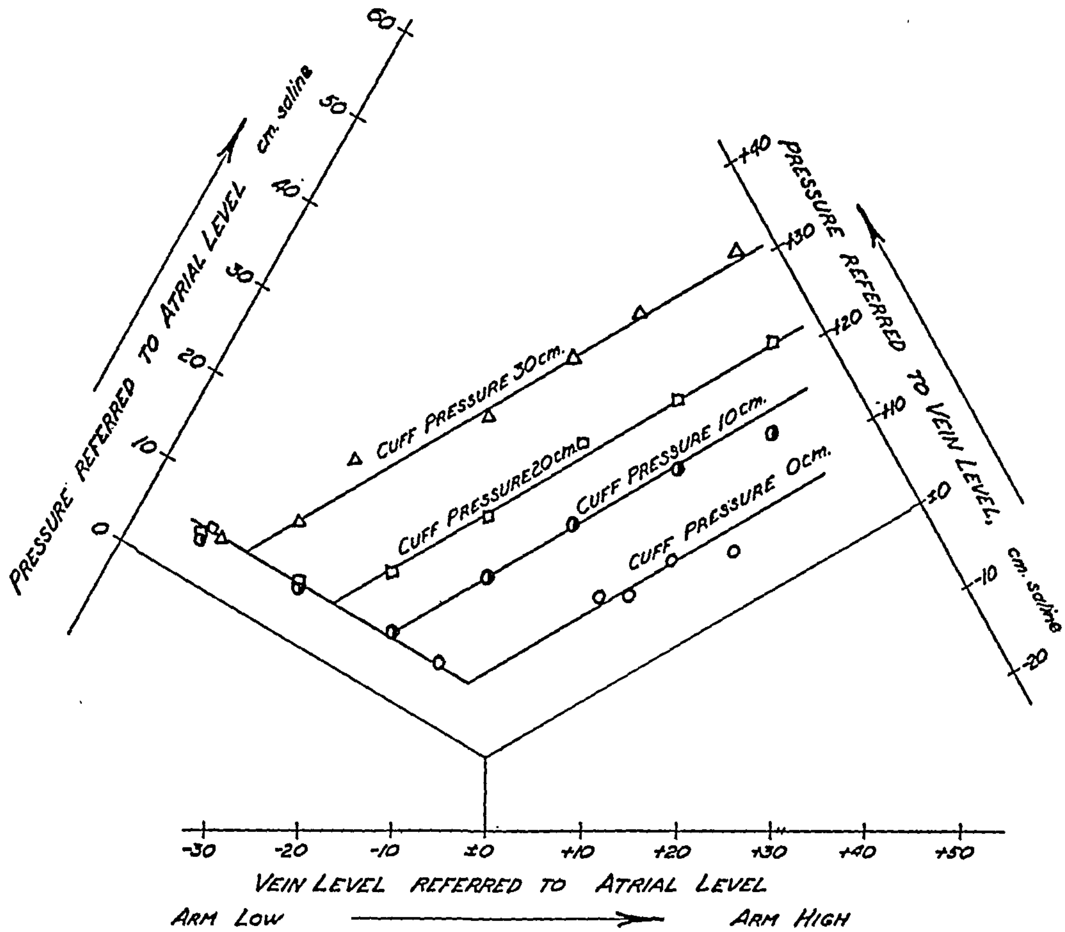

FIG. 9. PRESSURE RELATIONS IN THE SAME SUBJECT WITH DIFFERENT CUFF PRESSURES

The pattern is similar to the model (Figure 4). As long as the vein is distended, the pressure is constant when referred to the atrium, no matter what the vein level may be. When the vein is elevated sufficiently to collapse the vein, the internal pressure bears a constant relation to the particular cuff pressure applied at the time.

TABLE I

Values of tissue pressure as determined by two methods in two groups of normal individuals

There is no significant difference between the mean pressures for the two groups, as analyzed by "Students" "*t*" test (Fisher (8)).

Burch and Sodeman—Volar surface of forearm	Presented method—Dorsum of forearm			
mm. H_2O	*mm. H_2O*			
11	50	40	25	5
29	10	30	30	0
26	20	85	55	20
23	10	5	50	30
40	15	10	20	45
19	20	55	40	10
18	35	105	0	
17	65	5	5	
14	9	14	20	
39	11	11	25	

Method	Mean	S.E. of mean	Mean difference	*t*
Burch and Sodeman	23.60	±3.14	4.00	0.46
Presented	27.60	±3.98		

Significance: None

for the measurement of the pressures were different, the forearm was above the atrial level with the patient supine, in our group, and the forearm at atrial level with the patient sitting, in their group, and that their method is subject to restrictions in interpretation as outlined by McMaster (5), the fact that the two groups of measurements are of the same order of magnitude, and that statistically there is no significant difference between them, suggests that each method is measuring some function of the same variable, namely, the tissue pressure.

DISCUSSION

We have shown that under physiological conditions of flow, peripheral veins are freely collapsible tubes, and therefore have peculiar properties. When they are collapsed, the internal pressure has nothing to do with the pressure in the atrium or the pressure in the vein at any point central to the point of measurement.

This pressure can usually be shown to be equal to the tissue pressure around the vein by putting a cuff around the vein and demonstrating complete transmission of cuff pressure to the lumen. Furthermore, if the vein is in a freely collapsed state anywhere between the point of measurement and the atrium, even though it is distended at the point of measurement, the venous pressure is quite independent of the pressure in the atrium and depends instead on the pressure at the point of collapse.

The effect of the tissue pressure in collapsing a vein is additive to the effect of the level of the vein with respect to the central pressure head. The closer the vein level approaches the central pressure, the less the tissue pressure about the vein has to be in order to collapse it.

The importance of these characteristics in the interpretation of peripheral venous pressure is obvious. The influence of hydrostatic vein levels on peripheral pressure in veins has been emphasized by others (2, 6, 7). The influence of tissue pressure, which, in most *collapsed* arm veins, is the sole determinant of the venous pressure, has not previously been recognized to our knowledge.

SUMMARY

1. Under static conditions *in vitro*, veins and rubber tubes are freely distensible within wide limits. Within this range of free distensibility, the internal pressure is equal to the external pressure.

2. Under dynamic conditions, veins and rubber tubes are still freely distensible within wide limits.

3. Veins *in vivo* show the same pressure responses to central and local influences that they do *in vitro*.

4. A human arm vein *in vivo* can be determined to be within its range of free distensibility by elevating the arm until the vein appears collapsed, and then demonstrating the complete transmission to its lumen of pressure applied externally by means of a cuff.

5. When a vein is collapsed to within its limits of free collapsibility, the internal pressure is equal to the surrounding tissue pressure.

6. These findings place certain important restrictions on the interpretation of the venous pressure as measured peripherally.

CONCLUSIONS

When arm veins are distended, the venous pressure measures central influences, and is independent of the local pressure around the veins.

When arm veins are collapsed, the venous pressure measures the tissue pressure that has collapsed them, and is independent of central influences.

The measurement of local venous pressure in freely collapsed veins offers a means of measuring tissue pressure under conditions of equilibrium.

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PRESERVATION OF NORMAL HUMAN PLASMA IN THE LIQUID STATE. I. A STATISTICAL STUDY OF 1751 ADMINISTRATIONS[1]

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Of all the blood derivatives in use at present, plasma preserved in the liquid state is the simplest and most economical to prepare. It requires neither constant refrigeration, as does frozen plasma, nor an expensive desiccating set-up, as does dried plasma. Preservation in the liquid state has, however, certain distinct disadvantages. Probably the most important of these is the fact that relatively slight accidental bacterial contaminations during preparation may multiply to dangerous proportions during preservation. Others are the decrease in physiologic activity of labile constituents such as prothrombin, complement, and isohemagglutinins, studies on which are presented in the following papers (1, 2), the tendency of fibrinogen to precipitate out on storage, particularly at temperatures below 15° C., and the denaturation of proteins by temperatures above 37° C. It is chiefly the possibility of bacterial contamination, however, which has caused several authorities to advise against preservation of plasma in the liquid state (3).

For the past three years, the Plasma Department of the U. S. Naval Medical School has prepared plasma, preserved it in the liquid state, and distributed it to Naval Medical Activities within continental limits. It is the purpose of this communication to present a statistical study of the first 1751 complete reports of administrations of this plasma. It is proposed by this presentation to demonstrate that when normal human plasma is prepared by a closed system with scrupulously aseptic technique, and administered with suitable precautions, particularly with regard to filtration, it may be preserved in the liquid state for periods up to at least 15 months and transfused into patients with safety and benefit. Certain other aspects of plasma administration such as analyses of dosage, indications, and untoward reactions will also be presented.

METHODS

All the plasma administered in this study was prepared by the "closed vacuum" technique as described elsewhere (4, 5). In all lots, 50 per cent glucose and 1 per cent sodium ethyl mercuric thiosalicylate (merthiolate) were added so as to produce a final concentration of 5 per cent and 1 : 10,000 respectively. In the first 135 lots, the plasma was unpooled. In subsequent lots, the plasma from between 5 and 12 donors was pooled. The majority of the plasma was stored at room temperature throughout the study. This temperature ranged between 16° and 37° C. A small fraction was kept between 12° and 20° C. during the summer of 1941. The donors were all recruited by the District of Columbia Red Cross Volunteer Blood Donor Service and bled at the Army-Navy Blood Donor Center, Washington, D. C. The donors were asked to refrain from eating fatty foods for 6 hours previous to blood donation. The plasma was prepared in a laboratory located in the same building as the donor center. Plasma was issued by messenger, railway express, and air express to all continental Naval Medical Activities requiring plasma, upon request from these activities. Their geographic location ranged from Miami Air Station, Florida, to Bremerton Navy Yard, Washington. With the exception of the following instructions on the label of every bottle, no control was exercised over the administration of the plasma: "1. A filter must be used in the intravenous set. 2. Do not apply heat to plasma at any time, or to the intravenous equipment during administration. 3. Except in severe secondary shock, the rate of administration should not exceed 10 cc. per minute. 4. Plasma has a tendency to form a precipitate upon standing. This does not affect the safety or efficiency of the plasma." Every bottle was accompanied by a detailed questionnaire[4] for the collection of the information upon which this study is based. After February 1942, each shipment of plasma was accompanied by the instruction that it be stored at room temperature.

[1] This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the U. S. Navy. The opinions and views set forth in this article are those of the writers and are not to be considered as reflecting the policies of the Navy Department.

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[4] Space does not permit the inclusion of this questionnaire herein. It is available on application to the authors.

A code for transposition of the questionnaire to punch cards was drawn up. By means of an 80 column punch card, it was possible to code 19 items. Coding of all items involving a medical opinion such as indication or reaction was done by one of us (E.L.L.). Upon completion and proof-reading of the coding, punch card tabulations and counts and statistical analyses thereof were made by the Division of Vital Statistics, Bureau of Medicine and Surgery, Navy Department. All the data presented, therefore, are the results of questionnaire analysis.

RESULTS

A total of 516,140 cc. of citrated plasma was administered in this study to a total of 726 patients, giving an average dose in each administration of 295 cc. and an average amount given each patient of 711 cc. The upper limit of consecutive dosage to any individual was 3000 cc. and of total dosage was 12,600 cc. The distribution of total dosage is presented in Table I. It will be seen that because of the use of 250 cc. of plasma as the "unit" of dosage, the peak of the distribution comes between 200 and 299 cc. with a secondary peak between 500 and 599 cc. Similarly, Table II presents the distribution of number of administrations showing that approximately half the group required 2 or more injections and 15 per cent required 4 or more injections.

TABLE I

Total volume of plasma received by each patient

Volume	Number of patients	Volume	Number of patients
cc.		cc.	
100 to 199	7	2500 to 2599	1
200 to 299	246	2600 to 2699	2
300 to 399	44	2700 to 2799	1
400 to 499	64	2800 to 2899	2
500 to 599	155	2900 to 2999	1
600 to 699	12	3000 to 3099	2
700 to 799	37	3100 to 3199	1
800 to 899	22	3400 to 3499	1
900 to 999	21	3600 to 3699	1
1000 to 1099	28	3700 to 3799	1
1100 to 1199	4	4000 to 4099	1
1200 to 1299	12	4100 to 4199	1
1300 to 1399	6	4500 to 4599	2
1400 to 1499	3	4800 to 4899	1
1500 to 1599	10	5000 to 5099	2
1600 to 1699	4	5100 to 5199	1
1700 to 1799	4	5200 to 5299	1
1800 to 1899	7	6300 to 6399	1
2000 to 2099	5	7000 to 7099	1
2100 to 2199	2	7800 to 7899	1
2200 to 2299	2	10,400 to 10,499	1
2300 to 2399	2	12,600 to 12,699	1
2400 to 2499	2		
		516,140	726 Totals

TABLE II

Number of administrations received by each patient

Administrations	Patients	Administrations	Patients
1	385	11	2
2	174	12	4
3	60	14	1
4	33	15	2
5	21	16	1
6	12	18	1
7	6	20	4
8	6	21	1
9	5	34	1
10	6	50	1
		1751	726 Totals

Table III presents the distribution of indications for administration and the therapeutic results reported in each. It will be observed that the most frequent indication was hypoproteinemia and the next most frequent was

TABLE III

Clinical indication for administration and the therapeutic result reported

Clinical indications	Total	Result			Percentage of known group with beneficial results
		Beneficial	Not beneficial	Not stated	
Treatment of shock with hemorrhage	416	379	19	18	95.2
Treatment of shock without hemorrhage	136	111	13	12	89.5
Treatment of shock with burns	12	7	5	0	58.3
Treatment of shock with hemorrhage and burns	3	3	0	0	100.0
Treatment of hypoproteinemia	534	469	20	45	95.9
Treatment of hypoproteinemia associated with burns	47	45	0	2	100.0
Treatment of burns without shock	112	99	5	8	95.2
Prevention of shock with hemorrhage	298	271	3	24	98.9
Prevention of shock without hemorrhage	113	97	1	15	99.0
Prevention of shock with burns	1	1	0	0	100.0
Prevention of hypoproteinemia	9	6	1	2	85.7
None stated	70	51	1	18	98.1
Total	1751	1539	68	144	95.8
Per cent	100.0	87.9	3.9	8.2	

TABLE IV

Time of day plasma was administered

Time	Number	Per cent *
Midnight to 8:00 a.m.	144	8.8
8:01 a.m. to 4:00 p.m.	1094	67.3
4:01 p.m. to Midnight	388	23.9
Not stated	125	
Total	1751	100.0

* Omitting the group which did not state the time of administration.

shock in association with hemorrhage. It is of interest that almost one-fourth of the administrations were performed for reasons relating to the prevention of shock. Of the total groups of those in which a therapeutic result was reported, almost 96 per cent of the questionnaires stated the administration to be beneficial. It may be of significance that 8 per cent of the total group did not indicate any therapeutic result.

Table IV presents the distribution of administrations with respect to time of day. It will be observed that in this series, the nature of which has been previously defined, 67 per cent of the administrations were performed during the classical working day and only 9 per cent were performed between midnight and 8 a.m. Thus, in this series the administration of plasma would usually appear to be a procedure which one might classify as elective.

An intensive study was made of the administrations which were reported as being followed by untoward reactions. Some of the data concerning these administrations are presented in Tables V, VI, VII, VIII, and IX.

Seventy-two reactions in all were reported or an incidence of 4.1 per cent. Fifty-one were described as mild, 18 moderate, and 3 severe. Forty-nine were pyrogenic in type, *i.e.*, chills and/or fever, 13 urticarial, and 10 miscellaneous in character.

TABLE V

Distribution of administrations according to age of plasma and relationship to untoward reactions

Age of plasma	Total		Reactions		No Reaction		Reactions in each age group
months	*number*	*per cent*	*number*	*per cent*	*number*	*per cent*	*per cent*
0 to 0.9	37	2.1	4	5.6	33	2.0	10.8
1 to 1.9	226	12.9	14	19.4	213	12.7	6.2
2 to 2.9	238	13.6	10	13.9	228	13.6	4.2
3 to 3.9	263	15.0	15	20.8	248	14.8	5.7
4 to 4.9	219	12.5	8	11.1	210	12.5	3.7
5 to 5.9	223	12.7	5	6.9	216	12.9	2.2
6 to 6.9	168	9.6	7	9.7	164	9.8	4.2
7 to 7.9	142	8.1	7	9.7	134	8.0	4.9
8 to 8.9	51	2.9	0	0.0	51	3.0	0.0
9 to 9.9	32	1.9	0	0.0	32	1.9	0.0
10 to 10.9	46	2.6	2	2.8	44	2.6	4.3
11 to 11.9	50	2.9	0	0.0	50	3.0	0.0
12 to 12.9	23	1.3	0	0.0	23	1.4	0.0
13 to 13.9	22	1.3	0	0.0	22	1.3	0.0
14 to 14.9	9	0.5	0	0.0	9	0.5	0.0
15 to 15.9	2	0.1	0	0.0	2	0.1	0.0
Totals	1751	99.9	72	100.1	1679	100.1	4.1

Statistical evaluation

	Mean age of plasma	Standard deviation	
		Distribution	Mean
	months	*months*	
Total	5.02	2.98	0.07
Reactions	3.93	2.33	0.27
No reaction	5.06	3.01	0.07

The difference in age of plasma between that causing reactions and that causing no reaction is 1.1 months. The standard deviation of the difference is 0.28 months. $\frac{1.1}{0.28} = 3.9$. Therefore, the plasma causing reactions is significantly younger than that causing no reaction.

TABLE VI

Distribution of administrations according to hospitals and relationship to untoward reactions

Hospital number	Administrations	Reactions	Reactions
		number	*per cent*
1	55	2	3.6
2	70	3	4.3
3	1	0	0.0
4	150	14	9.3
5	215	12	5.6
6	9	0	0.0
7	326	14	4.3
8	33	0	0.0
9	12	0	0.0
10	398	12	3.0
11	16	2	12.5
12	11	0	0.0
13	21	1	4.8
14	3	1	33.3
15	17	0	0.0
16	75	1	1.3
17	53	2	3.8
18	27	1	3.7
19	10	0	0.0
20	11	1	9.1
21	2	0	0.0
22	2	0	0.0
23	45	0	0.0
24	144	1	0.7
25	19	0	0.0
26	4	0	0.0
27	7	2	28.6
28	15	3	20.0
Totals	1751	72	4.1

TABLE VII

Relationship of untoward reactions to clinical indication and distribution of types of untoward reactions

Indication	Total	No reaction	Number reactions	Percentage of reactions	Mild pyrogenic	Mild urticaria	Mild miscellaneous	Moderate pyrogenic	Moderate urticaria	Moderate miscellaneous	Severe urticaria	Severe miscellaneous
		Type of reaction										
Total	1751	1679	72	4.1	37	8	6	12	4	2	1	2
Treatment of shock with hemorrhage	417	405	12	2.9	6	1	1	1	2	1	0	0
Treatment of shock without hemorrhage	136	131	5	3.7	4	1	0	0	0	0	0	0
Treatment of shock with burns	12	12	0	0	0	0	0	0	0	0	0	0
Treatment of shock with hemorrhage and burns	3	3	0	0	0	0	0	0	0	0	0	0
Treatment of hypoproteinemia	534	511	23	4.3	10	1	3	5	0	1	1	2
Treatment of hypoproteinemia associated with burns	48	45	3	6.3	2	1	0	0	0	0	0	0
Treatment of burns without shock	110	105	5	4.5	2	0	0	2	1	0	0	0
Prevention of shock with hemorrhage	298	283	15	5.0	10	2	1	1	1	0	0	0
Prevention of shock without hemorrhage	113	108	5	4.4	3	1	0	1	0	0	0	0
Prevention of shock with burns	1	1	0	0	0	0	0	0	0	0	0	0
Prevention of hypoproteinemia	9	6	3	33.3	0	1	0	2	0	0	0	0
No apparent indication	70	69	1	1.4	0	0	1	0	0	0	0	0

TABLE VIII

Relationship of untoward reactions to rate of injection

Rate of injection	Reactions	No reaction
cc. per minute		
1 to 1.9	0	5
2 to 2.9	5	17
3 to 3.9	10	19
4 to 4.9	12	38
5 to 5.9	5	25
6 to 6.9	1	28
7 to 7.9	3	14
8 to 8.9	7	36
9 to 9.9	2	12
10 to 10.9	5	18
12 to 12.9	2	8
13 to 13.9	1	6
15 to 15.9	1	1
16 to 16.9	3	8
17 to 17.9	0	1
18 to 18.9	5	1
20 to 20.9	0	1
22 to 22.9	0	1
25 to 25.9	0	1
	62*	240

* Ten did not state time of injection.

Statistical evaluation

	Mean	Standard deviation Distribution	Standard deviation Mean
	cc. per minute	*cc. per minute*	
Reactions	7.82	4.87	0.62
No reaction	7.31	3.91	0.25

There is no statistically significant difference between the rate of injection of the plasma causing reactions and that causing no reaction.

Table V presents the relationship of age of preservation to the incidence of untoward reactions. It appears to be statistically significant in this series that the younger plasma is followed by reactions more often than the older plasma. In 744 administrations of plasma under 4 months old, there were 42 or 5.6 per cent reactions. In 1007 administrations over 4 months old, there were 30 or 3.0 per cent reactions. In 545 administrations over 6 months old, there were 14 or 2.6 per cent reactions. In 235 administrations over 8 months old, there were 2 or 0.9 per cent reactions.

Table VI presents the relationship of untoward reactions to the hospital concerned. This appears to be another significant factor in the production of untoward reactions. The reaction rate for various activities ranged from 0.0 to 33.3 per cent.

Table VII presents the relationship of untoward reactions to indication for administration. There does not appear to be any relationship except for a lower incidence (3.0 per cent) of reactions among those conditions in which shock was present than in those in which shock was absent (4.7 per cent).

Table VIII presents the relationship between rate of injection and appearance of untoward

reaction. Within the limits of this study, there did not appear to be any relationship.

In view of the fact that most of the plasma administered in this study was pooled, it was felt that the reactions could be divided into those possibly "attributable" to plasma and those probably "not attributable" to plasma, depending on whether or not other administrations from the same pool were satisfactory. It was decided arbitrarily that if two or more other administrations from the same pool were satisfactory, the reaction from a given administration was probably not "attributable" to the plasma. Table IX presents the results of this analysis. It will be observed that 52 of the 72 reactions fall into the group probably "not attributable" to the plasma. Thus, on this basis, only 1.1 per cent of the administrations could be said to be followed by reactions possibly "attributable" to the plasma.

Nine pools gave reactions on 2 administrations. No pool gave reactions on more than 2 administrations. Six of the 9 fell into the group probably "not attributable" to plasma as 2 or more other administrations from the same pool were satisfactory. In all 9, both reactions were at the same hospital. Three hospitals accounted for 14 of these 18 reactions. In 3 of the 9 pools, both reactions were by the same individual on

TABLE IX-A

Analysis of the untoward reactions with respect to "attributability" to plasma

Possibly "attributable" to plasma

Number	Lot number	Other administrations from same lot	Other satisfactory administrations from same lot	Type*
1	23	0	0	P
2	84	0	0	U
3	119	0	0	P
4	261.1	1	0	M
5	261.2	1	0	M
6	331.1	1	0	P
7	333.3	1	0	P
8	367.7	1	1	P
9	441.5	1	1	M
10	482.7	0	0	U
11	507.7	0	0	M
12	596.2	1	1	P
13	597.1	1	1	P
14	681.6	0	0	U
15	771.1	1	0	P
16	771.5	1	0	P
17	863.5	0	0	P
18	882.7	0	0	P
19	1326.3	0	0	P
20	875.5	1	1	P

* P = Pyrogenic, *i.e.*, chills and/or fever
U = Urticarial
M = Miscellaneous.

TABLE IX-B

Analysis of the untoward reactions with respect to "attributability" to plasma

Probably "not attributable" to plasma

Number	Lot number	Other administrations from same lot	Other satisfactory administrations from same lot	Type*
1	139.1	4	3	M
2	139.2	4	3	P
3	157.5	5	5	U
4	159.2	2	2	U
5	162.3	4	4	M
6	172.5	5	5	P
7	173.6	5	5	U
8	174.1	5	5	P
9	175.4	4	4	P
10	181.7	6	6	P
11	182.4	6	5	P
12	182.7	6	5	P
13	183.3	5	5	P
14	200.4	3	2	P
15	200.5	3	2	P
16	205.5	5	5	M
17	208.5	5	5	U
18	215.7	5	2	P
19	220.5	4	4	M
20	222.3	4	4	U
21	228.4	4	3	P
22	228.5	4	3	P
23	263.5	6	6	P
24	302.1	4	4	P
25	309.1	2	2	P
26	314.2	5	5	M
27	315.2	4	4	U
28	342.4	2	2	U
29	343.2	2	2	P
30	373.6	4	4	M
31	375.1	4	3	P
32	375.2	4	3	U
33	377.6	4	4	P
34	381.4	4	4	P
35	388.1	5	5	P
36	393.3	2	2	P
37	452.1	5	5	U
38	456.2	3	3	P
39	521.6	5	5	P
40	523.3	4	4	U
41	532.6	6	6	P
42	621.6	5	5	P
43	769.4	4	4	P
44	770.4	4	3	P
45	770.6	4	3	P
46	774.3	4	4	P
47	1000.2	3	3	P
48	1003.3	3	3	P
49	1055.3	3	3	P
50	1060.3	3	3	P
51	1311.1	3	3	P
52	1319.1	2	2	P

* P = Pyrogenic, *i.e.*, chills and/or fever
U = Urticarial
M = Miscellaneous.

the same day. Of these 18 reactions, only one was urticarial in nature. Thus, no pool gave urticaria to more than one patient. Of the 13 urticarias, 10 fell into the group "not attributable" to plasma. It appears, therefore, that urticaria is more frequently the result of recipient hypersensitivity to an allergen in the plasma than of the passive transfer of an antibody in the plasma causing the recipient to react to an allergen in his environment. Three of the urticarias occurred following the administration of plasma more than 7 months old indicating that the agent responsible was rather stable.

The 72 reactions were given by 63 individuals. No patient suffered more than 2 reactions. Thus, in this series, no patient could be said to be particularly susceptible to reactions from plasma. In the 9 patients who gave 2 reactions each, only 1 instance of urticaria occurred.

Of the 72 reactions, in 45 (62 per cent), the administration was reported as beneficial, in 7 (10 per cent), as not beneficial, and in 20 (28 per cent), the therapeutic result was not stated.

No instance of a hemolytic reaction was reported.

DISCUSSION

The findings presented in this study, coupled with those presented in the following papers (1, 2, 6), indicate that when plasma is prepared by a "closed" system with scrupulously aseptic technique, it may be preserved in the liquid state for many months and be administered with safety and benefit. The mean age of the plasma before administration was 5 months; 545 administrations were of plasma over 6 months old. The untoward reaction rate of this latter group was 2.6 per cent or less than half that of the group under 4 months old. In the group of 235 administrations over 8 months old, the reaction rate was 0.9 per cent. These figures appear to be statistically significant. Thus, on the basis of untoward reaction rate, it appears that we have not yet reached the upper limit for preservation of plasma in the liquid state. Most of the physiological activity of the labile constituents has completely disappeared within the first 4 months of preservation (1). Thus, after this period, one is left with a protein solution entirely satisfactory for the treatment of shock, burns, or hypoproteinemia, but unsatisfactory for the treatment of hypoprothrombinemia or infections. Since the former 3 conditions far outweigh the latter in military importance, preservation of plasma in the liquid state would be contraindicated for field use only by the limited range of temperature permissible. However, in the temperate zone, it is a satisfactory method of preservation, provided that the technique of preparation and bacteriologic control are exacting.

It is of interest to speculate upon the possible reasons for the decreased reaction rate upon prolonged storage. This phenomenon would appear to be related to the disappearance of some mildly toxic labile constituent. A comparable phenomenon has been described with respect to serum and is thought to be due to the occurrence of thrombin in fresh serum. Since most plasma contains a small amount of serum, due to small clots accidentally present in the bleeding, the same theory may be extended to explain our observations. Or it may be due to the disappearance of isohemagglutinins, as is described in a following paper (2). However, since it has not been demonstrated that the titer of isoagglutinins which occurs in blood plasma is toxic and it has been demonstrated that thrombin is toxic, the serum theory is more inviting. Still a third possibility is the "contamination" of fresh plasma by a labile pyrogen which then deteriorated on storage. At the present time, none of these theories can be absolutely proven or excluded.

The total reaction rate of 4.1 per cent is at the lower end of the range commonly observed for whole blood transfusions which varies from 1 per cent to 50 per cent (7), and was 9.7 per cent at the Mayo Clinic in 1943 (8). Undoubtedly, many of the 72 reactions reported resulted from the use of pyrogenic intravenous sets over which there was no control. Indirect evidence concerning this was obtained by virtue of the fact that most of the plasma was pooled and it was possible to determine in the case of each reaction whether or not other administrations from the same pool were satisfactory. In 52 of the 72 reactions, at least 2 other administrations from the same pool were satisfactory. It is felt, therefore, that only the remainder of the reactions, namely 1.1 per cent of the administration,

may be "attributable" to the plasma. The urticaria percentage of 0.7 per cent, is approximately the same as that which has been reported for whole blood (7).

SUMMARY AND CONCLUSIONS

1. One thousand seven hundred and fifty-one administrations of normal human plasma preserved in the liquid state have been tabulated and analyzed by the punch card system.

2. The average age of preservation was 5.0 months and 545 administrations were of plasma over 6 months old.

3. The total number of untoward reactions reported was 72. In 52 of these, at least 2 other administrations from the same pool were satisfactory. This leaves a reaction rate possibly "attributable" to plasma of 1.1 per cent.

4. Two factors appeared to influence the reaction rate significantly, the hospital concerned and the age of preservation; the longer the preservation, the fewer there actions within the limits studied. Possible reasons for the latter phenomenon are discussed.

5. It may be concluded that when normal human plasma is prepared by a closed system with scrupulously aseptic technique, and administered with suitable precautions, particularly with regard to filtration, it may be preserved in the liquid state at room temperature in the temperate zone for periods up to at least a year and administered to patients with safety and benefit.

6. It may be recommended that administrations of plasma preserved in the liquid state for more than a year be continued as there does not appear to be any contraindication to such administrations as a result of this study or any other studies to date.

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PRESERVATION OF NORMAL HUMAN PLASMA IN THE LIQUID STATE. II. COMPARATIVE *IN VITRO* STUDIES ON THE PHYSIOLOGIC ACTIVITY OF LABILE CONSTITUENTS OF LIQUID AND FROZEN PLASMA [1,2]

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The therapeutic use of human plasma is now well established (1 to 3). It is available in three preparations, liquid, frozen, and desiccated from the frozen state. The purpose of the present study is an evaluation of liquid and frozen plasma from the standpoint of the stability of certain constituents having important physiological activity.

METHOD

For the comparative study of liquid and frozen plasma, 3 fresh pools of plasma were prepared by the Blood Plasma Department of the U. S. Naval Medical School, Washington, D. C. One half of each pool was transferred to small containers holding 200 ml. of citrated plasma and immediately frozen at −30° C. The other half was kept in similar containers in the liquid state at room temperature. At predetermined intervals, a liquid sample and its duplicate frozen sample were shipped to Boston for study. The frozen sample was preserved in this state by shipment in solid carbon dioxide. The usual preparation of the plasma consisted of drawing blood into 35 ml. of 2.5 per cent sodium citrate to make a total volume of 300 ml. Thus, a 0.31 per cent sodium citrate concentration was obtained. Eighteen hundred ml. of plasma from such bleedings were aspirated after centrifuging into 200 ml. of 50 per cent glucose to give a volume of 2000 ml. This pool was then subdivided as given above. In one instance, the glucose was omitted to determine if the addition of this substance had any effect on the stability of the labile constituents.

No contamination of any of the samples was found on examination in Boston.

Each plasma was observed for its prothrombin, complement, non-protein nitrogen, total protein, albumin, globulin, fibrinogen, and free hemoglobin content, together with studies on its anti-hemophilic (plasma thromboplastin) properties, recalcification time, and pH. Changes in these various factors were measured over a period of 6 months.

HEMATOLOGICAL STUDIES

Platelet counts were performed by a direct method using a modified Rees-Ecker diluting solution (4). Normal values by this method vary between 200,000 and 400,000 platelets per c. mm. of whole blood.

Prothrombin concentration was estimated by determining the "prothrombin time" by a modification (5) of Quick's method (6). A "lyophilized" rabbit brain preparation (7) was used as the source of thromboplastin. Using this method, a "prothrombin time" of 25 seconds corresponds to a normal prothrombin concentration. From 80 to 100 per cent of the normal prothrombin concentration was considered to indicate no loss of prothrombin. It has previously been determined that dilution of plasma, to the extent obtained in the preparation of the plasma pools, did not alter the prothrombin concentration within the limits of experimental error.

Determination of anti-hemophilic property. The anti-hemophilic property of plasma was determined by comparing the coagulation time of 2 ml. of blood from a hemophilic subject, with the coagulation time of 2 ml. of the same blood to which 0.1 ml. of the plasma to be tested had been added. Coagulation times were performed by a modified (8) Lee and White method (9).

The same hemophilic subject was used for testing all samples of plasma. The coagulation time of this particular subject usually ranged between 30 and 60 minutes. A plasma containing a high concentration of anti-hemophilic factor reduced the coagulation time of this individual to a range between 10 and 15 minutes.

[1] The work described in this paper was done, in part, under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[2] This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the U. S. Navy. The opinions and views set forth in this article are those of the writers and are not to be considered as reflecting the policies of the Navy Department.

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The coagulation time of *normal* blood by the same method was 5 to 10 minutes.

Recalcification time. The recalcification time was determined by observing the time required for 1 ml. of plasma to clot on the addition of 0.2 ml. of 0.25 per cent calcium chloride. By this method, normal undiluted blood plasma will coagulate in 6 to 12 minutes.

Determination of plasma hemoglobin. The quantitative estimation of plasma hemoglobin was determined by the benzidine method of Bing and Baker (10, 11), modified by Shen and Ham (12).

Normal plasmas should show less than 10 mgm. of hemoglobin per 100 ml. of plasma by this method.

BACTERIOLOGICAL STUDIES

Culture.

(*1*) Plasmas were cultured aerobically in blood broth and on blood agar.

(*2*) Plasmas were also cultured anaerobically by the following method: 0.5 ml. of plasma was added to a tube of blood broth. The cotton plug in the tube was pushed down and a snug-fitting pledget of absorbent cotton then plugged in until it touched the regular stopper. Pyrogallic acid and sodium hydroxide were added above the pledget; the tube closed with a tight-fitting rubber stopper; and the culture incubated at 37° C.

Titration of complement. The complement titer was determined by observing the smallest amount of plasma which completely hemolyzed a 2 per cent suspension of sheep cells in the presence of 2 units of standard amboceptor. This amount of plasma was then considered to contain one unit of complement. The titer was reported as the number of such units per 1 ml. of the plasma.

Normal plasmas contained 5 to 15 units per ml., with the average varying from 10 to 15 units per ml.

CHEMICAL STUDIES

Estimation of the plasma pH was made by the glass electrode technique; non-protein nitrogen, by the method of Folin (13); total protein and its partition by a modification of the method of Howe (14). Fibrinogen was determined by a modification of the method of Cullen and Van Slyke (15). Normal values obtained by these methods are as follows: Non-protein nitrogen, 15 to 25 mgm.; total protein, 5.6 to 7.8 grams; albumin, 3.8 to 4.5 grams; globulin, 1.3 to 2.5 grams; and fibrinogen, 190 to 330 mgm. per 100 ml. of plasma. It should be noted that when fibrinogen was not determinable by the recalcification method, an attempt was made to obtain approximate values by the use of thrombin (16).

EXPERIMENTAL RESULTS

Plasma proteins

Over a period of 6 months, the changes in total plasma proteins in both liquid and frozen plasma were not remarkable. No material changes were found in the distribution of albumin or globulin. Fibrinogen, when it was possible to determine it, was present in normal amounts. As is shown in the protocol, fibrinogen could not be determined in the older liquid plasmas, due to failure of the coagulation mechanism. The clotting properties, when thrombin was used, were likewise impaired in some instances.

The non-protein nitrogen of the liquid or frozen plasma did not increase during a period of 6 months. These findings would indicate that both liquid and frozen plasma retained their full values as a source of plasma protein and had lost none of their effectiveness as blood substitutes from the thermodynamic standpoint.

Prothrombin

There was a marked difference between the prothrombin content of liquid and frozen plasma. Within 6 months, the prothrombin content of liquid plasma fell to less than 2 per cent of its original concentration. Frozen plasma still contained over 40 per cent of its prothrombin content at that time. In 2 instances, where the frozen plasma was thawed slowly over a period of 24 hours, the prothrombin content was reduced to 28 per cent of normal in contrast to plasma thawed rapidly where the prothrombin content was higher. It appears, therefore, that plasma stored in liquid form, for periods as long as 6 months, contains little or no prothrombin, while frozen plasma contains effective amounts of this constituent. Furthermore, it appears that rapid thawing of the frozen plasma is essential if the effective prothrombin content is to be maintained.

Glucose was added to the pooled plasma in 2 of the pools and omitted in one. The presence or absence of the glucose in no way altered the state of preservation of prothrombin.

Anti-hemophilic activity

The anti-hemophilic component of plasma, "globulin substance" or "plasma thromboplastin" of Howell (17), was remarkably well preserved in both liquid and frozen plasma. In fact, at the end of 6 months, when prothrombin was essentially absent in the liquid plasma, full potency of globulin substance appeared to be present.

PROTOCOL

	Pool No. 70								Pool No. 508								Pool No. 50			
	Liquid plasma				Frozen plasma				Liquid plasma				Frozen plasma				Liquid plasma—No glucose added			
Date	December 3, 1941	January 6, 1942	February 10, 1942	June 10, 1942	December 3, 1941	January 6, 1942	February 10, 1942	June 10, 1942	March 3, 1942	April 14, 1942	May 12, 1942	September 2, 1942	March 3, 1942	April 14, 1942	May 12, 1942	September 2, 1942	March 16, 1942	May 12, 1942	June 10, 1942	September 2, 1942
Bottle number	1	2	3	4	5	6	7		1	2	3		5	6	7	8	1	4	6	5
pH				7.3				7.4		7.4	7.3	8.1		7.6	7.5	8.1		7.1	7.1	8.2
Prothrombin concentration (*per cent of normal*)	46	15	8	Less than 2	60	27*	28*	45	60	10	14	Less than 2	70*	45*	65	40	40	20	17	Less than 2
Recalcification time amount of 0.25 per cent $CaCl_2$ added to 1 ml. (*ml.*)		0.2	0.2	0.2		0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Clotting time of plasma (*minutes*)		30	No clot	No clot		14	14	20	14	24	Some clot in 60	No clot	18	15	Some clot in 60	10	4½	Some clot in 60	Some clot in 180	No clot
Effect on hemophilic blood control coagulation time, 2 ml. hemophilic blood (*minutes*)	40	34	38	45	40	34	38	45	35	41	35	35	35	41	35	35	24	35	45	35
Coagulation time of 2 ml. hemophilic blood + 0.1 ml. plasma (*minutes*)	15	12	13	11	15	12	13	11	13	14	12	17	15	15	14	11	10	13	10	17
Platelets (*number per c. mm.*)	42,000	30,000	18,000	10,000	23,000	48,000	25,000	6,000	8,000	7,000	9,000		14,000	14,000	4,000		14,000	7,000	8,000	
Free hemoglobin (*mgm. per 100 ml.*)				3.8				8.6		Less than 0.1		2.0		Less than 0.1		3.0	7.8		7.5	8.6
Non-protein nitrogen (*mgm. per 100 ml.*)	24†	31†	30†	25†	25†	24†	28†	26†	27†	25†	25†	28†	26†	30†	29†	28†	30‡	30‡	32‡	31‡
Total protein (*grams per 100 ml.*)	6.7†	6.0†	6.9†	6.3†	7.5†	6.6†	6.8†	6.7†	6.5†	6.8†	5.6†	6.2†	6.7†	6.4†	7.2†	6.4†	6.5‡	6.1‡	6.4‡	6.8‡
Albumin (*grams per 100 ml.*)	4.3†	4.4†	4.4†	4.2†	4.5†	4.1†	4.3†	4.5†	3.8†	4.3†	3.9†	4.4†	4.0†	4.1†	4.7†	4.0†	4.2‡	3.8‡	4.3‡	4.4‡
Globulin (*grams per 100 ml.*)	2.2†	1.3†	2.3†	2.1†	2.7†	2.2†	2.2†	1.9†	2.5†	2.2†	1.4†	1.8†	2.3†	2.0†	2.2†	2.1†	2.0‡	2.0‡	1.9‡	2.4‡
Fibrin (*mgm. per 100 ml.*)	241†	253†	260†	Present but could not be determined	268†	260†	269†	252†	299†	291†	294†	None	320†	312†	344†	300†	266‡	231‡	227‡	None
Complement titration (*units per ml.*)		5	3	None		25	12	25	5	3	10	None		16	16	16	12	10	Less than 2	None
Culture	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Thawing time (*hours*)					½	24	24	3					24	24	2½	2½				

* Thawed slowly over 24 hours.
† Corrected for dilution with citrate and glucose, approximate correction × 1.4.
‡ Corrected for dilution with citrate, approximate correction × 1.26.

Recalcification time

As a test of the existence of a complete coagulation mechanism, samples of liquid and frozen plasma were recalcified. The liquid plasma was incoagulable in 2 to 6 months while the frozen plasma retained its clotting ability after the addition of calcium.

Complement

The data on complement titration indicate that this factor was well preserved in frozen plasma. In liquid plasma, it was lost within the 6 months' period of study.

Miscellaneous

The pH of both liquid and frozen plasma rose slightly from a normal value of 7.4 to approximately 8 over the 6 months' period. Counts of refractile bodies resembling platelets ranged from 4000 to 42,000 per c. mm. A decrease in the number was noted in some pools as time went on. This decrease was slightly more marked in the frozen than in the liquid plasma. Free hemoglobin never exceeded 10 mgm. per 100 ml. in any of the samples submitted to us. Cultures for bacterial growth were uniformly negative.

Rate of destruction of prothrombin

It would appear from the foregoing studies that the principal blood coagulation component which disappeared from liquid plasma was prothrombin. It seemed advisable, therefore, to study conditions under which the component could be stabilized.

Samples of whole blood were obtained and kept chilled until arrival in the laboratory. The cells were removed by centrifuging in a refrigerated centrifuge. Samples of plasma were removed under sterile conditions. One set of samples was kept in the icebox, the second set allowed to stand at room conditions. Samples of plasma were removed from each at daily intervals and tested for their prothrombin content and recalcification time. The results are shown in Figure 1.

It is evident that the prothrombin decreased very rapidly in the plasma kept at room temperature, as is well known. Actually, under the conditions of this experiment, in 10 days, there was virtually no prothrombin remaining, and at

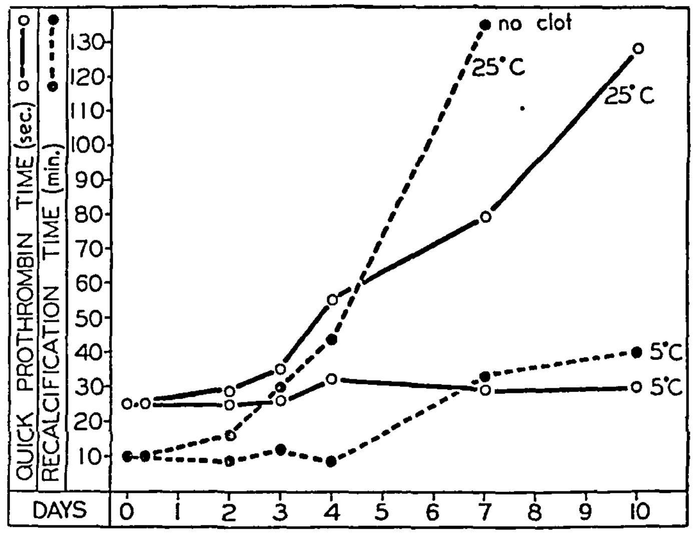

FIG. 1. THE EFFECT OF TEMPERATURE ON THE STABILITY OF PROTHROMBIN

the end of 7 days, the plasma was incoagulable on recalcification. On the other hand, plasma remaining in the icebox lost its prothrombin much more slowly, and the plasma still clotted in 40 minutes at the end of 10 days.

From the increase in recalcification time in the icebox experiment, it would appear that destruction of prothrombin is not the only factor responsible for the loss of the property of coagulability. It has been our experience that changes in recalcification time of normal plasma seldom occur unless the prothrombin is reduced below 10 per cent of normal. In the experiment reported here, the prothrombin concentration remained at 45 per cent of its normal value and yet the recalcification time of the plasma kept in the icebox rose from 10 minutes to 40 minutes.

While the actual determinations shown in the figure were all made after adjustment to pH 7.4, nevertheless the pH of the plasma kept in the icebox had in 10 days risen to 8.7, and that at room temperature, to 9.0.

DISCUSSION

Lozner and Newhouser (18), in the preceding paper, report that liquid plasma may be administered with minimal untoward reactions, even when preserved at room temperature for over a year. Furthermore, it was observed, from a statistical analysis of the reactions encountered that they became more infrequent as the plasma was aged. The relative ease and

the low cost of manufacture of liquid plasma and the infrequency of reactions demand a complete appraisal of this therapeutic agent.

The data in the paper immediately following (19) indicate that even after storage in the liquid form for as long as two years, there is minimal protein degradation. It is of interest therefore that the present studies indicate that the physiological activity of prothrombin, and possibly other components of the blood coagulation reaction together with complement, have completely vanished within 6 months. Frozen plasma on the other hand retains such activity.

In evaluating these results with regard to liquid plasma as a therapeutic agent, several considerations are involved. These relate, first of all, to the indications for which the plasma is to be administered and, secondly, to those factors in the plasma which might be concerned with such indications. By far the leading indications for plasma administration are the prevention and treatment of shock resulting from plasma loss following trauma or burns, and the prevention and treatment of hypoproteinemia of various origins. The factors in the plasma concerned with these indications are, primarily, those concerned with the maintenance of colloid osmotic pressure and, secondarily, those concerned with nutrition, coagulation, and immunity. The composition of liquid plasma is adequate for the first indication. With regard to the latter three factors, the present publication deals only with *in vitro* studies. Certainly these are sufficiently clear-cut to warrant the statement that in patients with hypoprothrombinemia, hemorrhagic diathesis, or infections, the use of stored liquid plasma could not be expected to have any specific therapeutic effect, due to absence of prothrombin, coagulation effect, and complement. The present tendency toward large dosage in burns and trauma may have a significant influence in the final decision on the disadvantage of the use of liquid plasma. One cannot deny that there is a theoretical hazard in administering large amounts of plasma, devoid of coagulation properties and complementary activity. The answer as to whether this hazard is more theoretical than real will depend on extensive clinical trial and investigation under conditions where these properties are required. Similarly, the nutritional value of such plasma must await long-term balance studies.

SUMMARY

1. A study of the stability of certain labile components of plasma preserved in the liquid and frozen state has been made.

2. The protein components of both liquid and frozen plasma are well preserved.

3. The coagulation factors of liquid plasma are lost but better preserved in frozen plasma.

4. Complement is lost from liquid plasma but preserved in frozen plasma.

5. The effect of temperature on the preservation of prothrombin is indicated.

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PRESERVATION OF NORMAL HUMAN PLASMA IN THE LIQUID STATE. III. STUDIES ON CHEMICAL AND PHYSICO-CHEMICAL CHANGES DURING THE SECOND YEAR OF STORAGE [1,2]

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The present regulations of the National Institute of Health with regard to the preservation of plasma in the liquid state call for an expiration date of one year. In the first paper of this series (1), 235 administrations of plasma preserved in the liquid state at room temperature for between 8 and 16 months were performed with an untoward reaction rate of 0.9 per cent and with the expected proportion of beneficial therapeutic results. The second paper of this series (2) has indicated that except for an inability to determine fibrinogen in plasma preserved in the liquid state for 6 months, there were no significant chemical changes in the total protein, albumin-globulin ratio, or non-protein nitrogen content of plasma. The clinical innocuousness of plasma preserved for periods longer than 6 months made of interest the investigation of what chemical and physico-chemical changes might be taking place during storage of such duration.

METHODS

Eighteen samples of 11 pools of plasma, prepared by the Blood Plasma Department of the Naval Medical School and preserved in the liquid state at room temperature in Washington, D. C., for between 15 and 24 months, were obtained for chemical and physico-chemical studies. The method of plasma preparation has been described previously (1). Six additional samples of lots, stored for less than a week, served as controls.

Total protein was measured by a modification of the method of Keys (3); albumin-globulin ratio by a modification of Howe's method (4). Fibrinogen determination was attempted unsuccessfully by the recalcification method (5), the salting-out method (6), and the thrombin method (7). Non-protein nitrogen was determined by Keys' method, 3 protein precipitants being employed on aliquots of each sample, 10 per cent trichloroacetic acid, 2.5 per cent trichloroacetic acid, and tungstic acid. The interpretation of the contents of the filtrates was made according to the principles laid down by Hiller and Van Slyke (8). By these principles, the difference between the nitrogen content of the 2.5 per cent trichloroacetic acid filtrate and that of the tungstic acid filtrate is an index of the polypeptide content of the plasma. A wide range of values has been reported for this index in normals (9, 10). The limits of this range are stated in Table II. The residual nitrogen was calculated according to Berglund (11) and the range of normal given in Table II is his. Urea was determined by the urease manometric method of Van Slyke (12). Free amino-acids were measured by determining the α-amino nitrogen by the ninhydrin-carbon dioxide gasometric method of Hamilton and Van Slyke (13 to 15). Osmometric determinations were made by Davis' (16) modification of the Hepp apparatus (17). Electrophoretic patterns were done by Dr. H. Kahler of the National Cancer Institute, using the Longsworth modification (18) of the Tiselius apparatus (19).

RESULTS

The chemical findings are presented in the accompanying tables. All findings are presented corrected for the initial dilution by sodium citrate and glucose.

It will be observed from Table I that no gross changes in the chemical determinations of total

[1] This article has been released for publication by the Division of Publications, of the Bureau of Medicine and Surgery of the U. S. Navy. The opinions and views set forth in this article are those of the writers and are not to be considered as reflecting the policies of the Navy Department.

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TABLE I

Nitrogen components of pooled human plasma, preserved in the liquid state at room temperature for from 15 to 24 months

Nitrogen components	Normal	Preserved liquid plasma (18 samples)	
	Range	Range	Average
	grams per 100 ml. of plasma		
Total protein	6.3 to 8.0	6.0 to 7.2	6.5
Albumin	3.7 to 5.3	3.8 to 5.5	4.5
Globulin	1.9 to 3.6	1.6 to 3.4	2.3

protein, albumin, or globulin appear to be taking place during the second year of storage. It was impossible to determine fibrinogen by any of the chemical methods for reasons previously explained (2). There is a vague suspicion that the albumin concentrations were running a little higher than expected, and the globulins, a little lower. This is to be borne in mind in connection with the physico-chemical studies discussed below.

Table II presents the findings with respect to the non-protein nitrogen and certain of its components. All of the urea concentrations fell within normal limits. However, the total non-protein nitrogen and the amino-acid nitrogen both increased slightly on prolonged storage. The former, of course, varied with the protein precipitant used as is discussed below, but in general was 10 to 20 mgm. per 100 ml. higher than normal. The α-amino nitrogens in preserved plasma ran from 5 to 10 mgm. per 100 ml. higher than the controls.

The "residual" nitrogen, that is, the non-protein nitrogen less the α-amino and urea nitrogen, also increases slightly on prolonged storage. The significance of this increase was investigated by means of a determination of the "polypeptide index," the difference in nitrogen content between a tungstic acid filtrate and a 2.5 per cent trichloroacetic acid filtrate. The rationale for this "index" is that the tungstic acid precipitates polypeptides whereas this concentration of trichloroacetic does not (8). The polypeptides, by this method, also appear to increase slightly on storage. They ran from 3 to 10 mgm. per 100 ml. greater than normal. Thus, the increase in non-protein nitrogen would appear to be made up of approximately equal parts of amino-acid and polypeptide nitrogen. Preliminary data utilizing hydrolysis of the protein-free filtrate are confirmatory of this conclusion.

Of these components of the non-protein nitrogen, thus far, only the α-amino nitrogen has been partially correlated with age. It will be observed from Table III that even here the correlation is not perfect.

TABLE II

Non-protein nitrogen components of pooled human plasma, preserved in the liquid state at room temperature for from 15 to 24 months

Non-protein nitrogen components		Normal	Preserved liquid plasma (18 samples)	
		Range	Range	Average
		mgm. per 100 ml. of plasma		
Urea-N		10 to 17	11.3 to 16.5	14.2
α-amino-N (Ninhydrin-Carboxyl)		4 to 6	8.8 to 13.9	10.5
NPN	Filtrates			
	10 per cent CCl_3COOH	18 to 30	32.3 to 49.7	40.1
	2.5 per cent CCl_3COOH	20 to 33	35.7 to 51.1	44.1
	Tungstic Acid	18 to 30	30.9 to 43.7	35.6
"Polypeptide Index" NPN(2.5 per cent CCl_3COOH)-NPN (Tungstic Acid)		3 to 8	3.2 to 14.8	8.4
"Residual" Nitrogen NPN(10 per cent CCl_3COOH)-(Urea-N + Amino-Acid N)		2 to 12	10.5 to 23.2	15.3

TABLE III

Correlation of α-amino nitrogen with age in pooled human plasma, preserved in the liquid state

Age of plasma	α-amino Nitrogen	
	Range	Average
	mgm. per 100 ml. of plasma	
5 days (Control) (3 samples)	4.9 to 5.7	5.2
15 months (12 samples)	8.8 to 11.0	9.9
18 months (1 sample)	10.2	10.2
19 months (1 sample)	11.8	11.8
20 months (3 samples)	11.5 to 14.0	12.5
24 months (1 sample)	12.1	12.1

DISCUSSION

The 6.3 to 8.0 grams of protein in 100 ml. of normal plasma represent from 1000 to 1280 mgm. of nitrogen of which from 700 to 1000 mgm. is α-amino nitrogen (13) upon proper hydrolysis. Thus, the increase of from 5 to 10 mgm. of α-amino nitrogen, such as was observed here upon storage of plasma in the liquid state for 2 years, represents hydrolysis to this degree of only 0.5 to 1.0 per cent of the original protein.

The highest non-protein nitrogen observed, namely 51.1 mgm. per 100 ml., indicates hydrolysis to non-protein size of only 2 per cent of the original protein. Thus, from this chemical point of view, 98 to 99.5 per cent of the original precipitable protein can be said to have remained intact. However, gross cleavages of protein may occur without significant increase in α-amino or non-protein nitrogen as long as the molecules remain large enough to be precipitable by the usual reagents. Chemical methods, such as the albumin-globulin ratio, have failed to demonstrate conclusively the occurrence of such cleavages in this study. So it has been necessary to turn to physico-chemical methods to clarify this point. Thus far, these data are too incomplete to report in detail. Upon completion, they will be reported in a subsequent paper (20). However, the preliminary electrophoretic analyses and osmometric determinations suggest that such cleavages are, to a certain extent, taking place. The electrophoretic mobilities of the globulin fraction are nearer and invade somewhat the albumin component and the plasma as a whole seems somewhat more effective osmotically than fresh plasma. These changes, however, would not detract from the use of such plasma for its colloid, for example, as an anti-shock agent or in hypoproteinemia, in situations where the labile constituents are unimportant. In fact, if subsequent observations confirm the greater osmotic effectiveness of preserved plasma and fewness of untoward reactions, storage in the liquid state may be most desirable for such indications.

It is of interest to speculate upon the nature of the proteolytic enzyme which may be responsible for the changes observed here. An enzyme resembling trypsin has been prepared from plasma treated with chloroform (21, 22). Inasmuch as in preserved plasma, both free amino-acids and polypeptides are increased, the enzyme operating must contain both proteinase and peptidase activity. Therefore, it is not identical with crystalline trypsin of Northrup and Kunitz which does not contain any peptidase activity (15). In its activity at the pH of plasma, 7.4, it does resemble the enzyme of chloroform-treated plasma which has optimal activity at this pH (22).

SUMMARY AND CONCLUSIONS

1. Chemical findings on 18 samples of plasma, preserved in the liquid state for 15 to 24 months at room temperature, are presented.

2. During storage of such duration, the non-protein nitrogen content, the α-amino nitrogen, the "residual" non-protein nitrogen, and the "polypeptide index" all increased slightly.

3. No gross chemical changes can be detected in the total protein, albumin, or globulin content but it appears from the increase in amino-acids and polypeptides that from 0.5 to 2.0 per cent of the original protein is hydrolysed to molecules of these sizes. Therefore, 98 to 99.5 per cent of the original protein remains precipitable.

4. Preliminary electrophoretic and osmometric data indicate that a limited amount of protein cleavage takes place. The osmotic effectiveness of the preserved plasma appears to be slightly increased over fresh plasma.

5. In view of the above findings, it may be concluded that when plasma is prepared by a "closed" system, with scrupulously aseptic technic and careful bacteriologic control, it may be preserved in the liquid state at room

temperature in a moderate climate for periods up to at least two years.

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PRESERVATION OF NORMAL HUMAN PLASMA IN THE LIQUID STATE. IV. STUDIES ON ISOHEMAGGLUTININ TITERS[1]

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The presence of isohemagglutinins in plasma has prompted at least two observers to attribute untoward reactions following the administration of *pooled* plasma to this factor (1, 2). While it is conceivable that in plasma from a *single* donor, the same hazard may theoretically exist as in blood from a "dangerous universal donor," it is nevertheless a fact, as has been pointed out by Thalhimer (3), that for purposes of bacteriologic control, the preparation of plasma on a large scale invariably involves pooling. It is the purpose of this communication therefore to report isohemagglutinin titrations on 1000 pools of plasma as compared with 100 monovalent controls and to present a preliminary investigation of the clinical significance of the isoagglutinin titers of pooled plasma.

METHODS

One thousand specimens of pools of plasma, prepared by the Blood Plasma Department of the U. S. Naval Medical School, were analyzed for their titer of isohemagglutinin against "A" and "B" red cell suspensions. The method of plasma preparation has been described previously (4). The method of titration was that in which 0.5 ml. of plasma was diluted geometrically with 0.9 per cent sodium chloride in a rack of 10 × 75 mm. test tubes. To each dilution is added 0.5 ml. of a 1 per cent washed red cell suspension from a panel of "A" and "B" individuals. The tubes are allowed to stand without agitation for 2 hours at 37° C. Following this, each tube is gently agitated and the end-point is read as the last tube in which macroscopic agglutinates are present. The first tube, *i.e.*, that in which 0.5 ml. undiluted plasma has been added to 0.5 ml. red cell suspension, is called "1 : 2" inasmuch as a 50 per cent dilution of the plasma is present. All titrations were done in duplicate and each time a series of titrations was run, controls consisting of plasma from a known "A" and a known "B" individual were also run. In all, 100 "A" and 100 "B" monovalent control titrations were done. The pooled plasma varied with respect to age of preservation in the liquid state and to the number of donors of the pool.

In connection with the statistical study of blood plasma administration presented in the first paper of this series (5), it was possible to compare the distribution of agglutinin titers in those lots of plasma which had been reported as being followed by untoward reactions with a sample of those lots of plasma which had been reported as not followed by reactions. It was also possible to determine the reaction percentage in the administration of those lots of plasma which turned out to have agglutinin titers over "1 : 16."

RESULTS

The distribution of isohemagglutinin titers in 1000 pools of plasma and 100 monovalent controls and the correlations with the age of plasma and with the number of donors contributing to the pools are presented in the accompanying tables (Tables I and II).

It will be observed that pooling alone reduces the percentage of the group with titers of "1 : 16" and over from 72 per cent in the controls to 7.7 per cent in the pools, with respect to anti-A agglutinin, and from 78 per cent in the controls to 15.2 per cent in the pools, with respect to anti-B agglutinin.

The difference between pools with only 5 to 8 donors contributing to them and those with 9 to 12 donors is striking. Whereas the former group had percentages of titers of "1 : 16" and over, of 8.1 per cent and 15.7 per cent of anti-A and anti-B agglutinin, respectively, the latter had percentages of 1.5 per cent and 8.7 per cent.

It is also evident that aging the plasma in the liquid state over 4 months also decreases considerably the percentage of the group with titers of "1 : 16" and over. In 145 pools over 4 months old, none had a titer of "1 : 16" or over of anti-A agglutinin and 7 had titers of "1 : 16" to "1 : 64," but none over "1 : 64," of anti-B

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agglutinin. Between the group under 1 month old and the group 1 to 4 months old, however, no significant difference could be elicited.

The clinical significance of the agglutinin titers in pooled plasma was investigated by means of two approaches as described above. Thus far, 15 administrations of pools with titers known to be over "1 : 16" have been performed without untoward reaction. In addition, no correlation could be elicited between titer and untoward reactions as tabulated in the statistical analysis of the first paper of this series (5).

DISCUSSION

The data presented here tend to discredit the reports (1, 2) that untoward reactions may be due to the titer of agglutinins present in pooled plasma. The reaction reported by Polayes and Squillace (1) was ascribed by them to the presence of a "1 : 8" titer of agglutinins. In the thousand pools studied here, 17.7 per cent had a titer of "1 : 8" or over of anti-A agglutinin and 28.6 per cent of anti-B agglutinin. Yet there was no increase in untoward reaction rate in this group. If the premise of Polayes and Squillace were correct, practically no type "O" blood could be transfused into a heterologous patient. Thousands of such transfusions have been and are being performed with no greater than the usual percentage of untoward reactions. A titer of "1 : 8" would be innocuous on the basis of dilution alone in the recipient's bloodstream and there appear to be factors in addition to dilution which enable patients to tolerate incompatible agglutinins.

The reaction reported by Downs (2) was typically urticarial, quickly responding to adrenalin. Nevertheless, he ascribed it to an incompatibility between the plasma and the patient's blood, although no test for such incompatibility was performed, and he advocated preliminary cross-matching before every administration of plasma. Our data indicate that such cross-matching would show the presence of small concentrations of agglutinins about 50 per cent of the time. Such concentrations of agglutinins cannot be

TABLE I

Analysis of anti-A isoagglutinin titration on 1000 pools of plasma

Groups		0	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	Total
Monovalent controls	Number	0	0	5	23	25	21	17	4	4	1	100
	Per cent	0	0	5	23	25	21	17	4	4	1	100 per cent
						72 per cent						
Total pools	Number	551	144	128	100	48	14	12	3	0	0	1000
	Per cent	55.1	14.4	12.8	10.0	4.8	1.4	1.2	0.3	0	0	100 per cent
						7.7 per cent						
Pools with 5 to 8 donors	Number	510	133	120	92	47	14	12	3	0	0	931
	Per cent	54.8	14.3	12.9	9.9	5.0	1.5	1.3	0.3	0	0	100 per cent
						8.1 per cent						
Pools with 9 to 12 donors	Number	41	11	8	8	1	0	0	0	0	0	69
	Per cent	59.4	15.9	11.6	11.6	1.5	0	0	0	0	0	100 per cent
						1.5 per cent						
Pools under 1 month old	Number	284	93	91	70	29	7	11	0	0	0	585
	Per cent	48.5	15.9	15.6	12	5.0	1.1	1.9	0	0	0	100 per cent
						8 per cent						
Pools 1 to 4 months old (Liquid)	Number	156	34	29	21	19	7	1	3	0	0	270
	Per cent	57.8	12.6	10.7	7.8	7.0	2.6	0.4	1.1	0	0	100 per cent
						11.1 per cent						
Pools over 4 months old (Liquid)	Number	111	17	8	9	0	0	0	0	0	0	145
	Per cent	76.6	11.7	5.5	6.2	0	0	0	0	0	0	100 per cent
						0 per cent						

TABLE II

Analysis of anti-B agglutinin titrations on 1000 pools of plasma

Groups		0	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	Total
Monovalent controls	Number	0	0	4	18	32	25	10	5	4	2	100
	Per cent	0	0	4	18	32	25	10	5	4	2	100 per cent
						78 per cent						
Total pools	Number	329	187	198	134	92	42	13	2	2	1	1000
	Per cent	32.9	18.7	19.8	13.4	9.2	4.2	1.3	0.2	0.2	0.1	100 per cent
						15.2 per cent						
Pools with 5 to 8 donors	Number	299	171	185	130	90	40	11	2	2	1	931
	Per cent	32.1	18.4	19.9	14	9.7	4.3	1.2	0.2	0.2	0.1	100 per cent
						15.7 per cent						
Pools with 9 to 12 donors	Number	30	16	13	4	2	2	2	0	0	0	69
	Per cent	43.5	23.2	18.8	5.8	2.9	2.9	2.9	0	0	0	100 per cent
						8.7 per cent						
Pools under 1 month old	Number	148	113	130	93	59	27	10	2	2	1	585
	Per cent	25.3	19.3	22.2	15.9	10.2	4.6	1.7	0.3	0.3	0.2	100 per cent
						17.3 per cent						
Pools 1 to 4 months old (Liquid)	Number	92	52	50	32	28	14	2	0	0	0	270
	Per cent	34.1	19.3	18.5	11.8	10.4	5.2	0.7	0	0	0	100 per cent
						16.3 per cent						
Pools over 4 months old (Liquid)	Number	89	22	18	9	5	1	1	0	0	0	145
	Per cent	61.4	15.2	12.4	6.2	3.4	0.7	0.7	0	0	0	100 per cent
						4.8 per cent						

demonstrated to be harmful and it may be concluded therefore that preliminary cross-matching of *pooled* plasma does not appear to be justified experimentally.

SUMMARY AND CONCLUSIONS

1. An analysis of isohemagglutinin titrations on 1000 pools of plasma and 100 monovalent controls is presented.

2. Pooling the plasma reduced the percentage with titers of "1 : 16" and over, of anti-A isoagglutinin from 72 per cent in the controls to 7.7 per cent in the pools, and of anti-B isoagglutinin from 78 per cent in the controls to 15.2 per cent.

3. Pools with 9 to 12 donors have a lower percentage of titers of isoagglutinins of "1 : 16" and over than pools with 5 to 8 donors.

4. Pools over 4 months old have a much lower percentage of titers over "1 : 16" than pools under 4 months old.

5. To date, 15 administrations of plasma with titers over "1 : 16" have been performed with- significant difference in titers of isoagglutinin in lots of plasma reported as being followed by untoward reactions, compared with lots of plasma which were reported as not followed by reactions.

6. It may be concluded that thus far there is no evidence for any harmful effect from the titer of isohemagglutinins present in *pooled* plasma, and, consequently, no justification for preliminary cross-matching of such plasma prior to administration.

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THE EFFECT OF A SINGLE INJECTION OF CONCENTRATED HUMAN SERUM ALBUMIN ON CIRCULATING PROTEINS AND PROTEINURIA IN NEPHROSIS[1]

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The intravenous injection of concentrated human serum or plasma into patients with hypoproteinemia of the nephrotic syndrome has not generally produced the desired rise in serum protein concentration (1 to 3). Diuresis and loss of edema are usually disappointing and rarely permanent.

Study of the serum proteins and hematocrit (2, 3) would seem to indicate that the injected plasma was soon withdrawn from the circulation, with little effect on the original serum albumin or globulin concentration. Not more than half of the injected protein appeared in the urine. In dogs with nutritional edema, injected protein was quickly removed from the circulating blood without any loss of protein in the urine (4).

In order to study the movements of protein and fluid, a single injection of concentrated human serum albumin has been given to 3 patients suffering from the nephrotic syndrome. Since this material is electrophoretically homogeneous, the injected protein can be traced when sufficiently large amounts are injected and when the plasma volume is determined concurrently.

MATERIAL AND METHODS

The purified human serum albumin was prepared in the Plasma Fractionation Laboratory of the Harvard Medical School.[1] The method earlier in use there for the preparation of this fraction was developed by Cohn, *et al.* (5).

Plasma protein determinations were made by Kjeldahl nitrogen determination. Urine protein was determined by dry weight.

The electrophoresis apparatus of Tiselius as modified by Longsworth (6) was used to study the protein distribution. With cloudy serum, large variations of photographic exposure on the recording plate displace the light-shadow boundary unequally and lead to considerable error. The use of a horizontal slit instead of a single knife-edge for scanning deflection of light avoids this error, since the recorded diagram is then outlined by a narrow line of exposure which is not displaced by over- or under-exposure of the photographic plate.

The buffer used in the electrophoresis of serum affects the relative mobilities of the protein fractions (6). The fraction described in a previous report (7) as α-globulin, appearing in phosphate buffer of ionic strength 0.2 and pH 7.8, is the same as Tiselius α-globulin. The fraction described in the present study as α_1 appears just behind the albumin boundary, in veronal buffer of ionic strength 0.1, at pH 8.6, and is apparently not the same as Tiselius' α-globulin, which cannot be accurately differentiated from the large β-globulin peak in nephrotic serum in the veronal buffer.

The plasma volume was determined with the blue dye T-1824, with suitable corrections for the diminution in the cloudiness of the serum which follows the injection of serum albumin. If such a correction is not applied, the fall in optical density of the serum indicates an erroneously high dilution of the dye. An equation for the corrected L620 can be set up in the same form as the usual correction for hemolysis but with different constants. If no hemolysis is present, the optical density at 540 mμ may be used, but it may be preferable to use a wave length less sensitive to traces of hemoglobin. The ratio of optical density at 620 mμ to that at the indifferent wave length is independent of dilution, both for dye and for cloudiness, within the usual range. The ratios may therefore be considered as constants and substituted in a general equation as follows:

$$\frac{\text{L620}}{\text{L540}} \text{ due to cloudiness} = K \text{ (determined from blank serum)}$$

$$\frac{\text{L620}}{\text{L540}} \text{ due to dye} = K' \text{ (determined from dye)}$$

$$\text{L620 due to dye} = \frac{\text{Observed L620} - (K \times \text{observed L540})}{1 - (K/K')}$$

The colloid osmotic pressure of the serum was determined with the Hepp osmometer (8, a. and b.).

All patients were young adults with an insidious onset of edema, presenting the characteristic features of the nephrotic syndrome. Patients I and II were obviously edematous, but patient III seldom had perceptible edema on a salt-free diet. In all cases at the time of the study, the heart was normal, blood pressure was not elevated, and there was no reduction in phenolsulfonphthalein excretion, urea clearance, or concentrating power of the kidney. On other occasions, patients I and III had some hypertension and microscopic hematuria, but these manifestations were never observed in patient II.

[1] The concentrated human serum albumin used in this work was produced under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research Development and Harvard University.

TABLE I
Electrophoretic analysis of proteins of serum and urine

Case	Time	Fluid	Albumin	α_1	Globulins $\alpha_2+\beta$	Globulins γ
			per cent		*per cent*	
I	Control	serum	13.0	7.2	74.2	5.6
	Control day I	urine	46.1	20.4	20.5	13.0
	Control day II	urine	46.4	20.5	21.2	11.9
	Post-injection	serum	30.1	5.7	59.9	4.3
		urine	70.7	10.3	13.2	5.8
	2nd day	urine	58.5	15.4	17.9	8.2
	3rd day	serum	15.0	6.4	73.0	5.6
		urine	55.3	15.7	18.8	10.2
II	Control	serum	17.2	3.9	72.5	6.4
		urine	65.3	11.2	15.0	8.5
	Post-injection	serum	23.9	2.5	66.9	6.7
		urine	79.9	6.9	8.7	4.5
	2nd day	serum	20.0	4.2	68.0	7.8
		urine	75.4	8.7	9.7	6.2
	3rd day	serum	18.6	4.0	69.5	7.9
		urine	67.5	12.5	12.2	7.8
III	Control	serum	15.0		79.4	5.6
		urine	62.6	14.4	19.3	3.7
	Post-injection	serum	29.2		66.1	4.7
		urine	90.8	4.6	3.6	1.0
	2nd day	serum	19.1		75.5	5.4

RESULTS

In control observations, all patients had a large initial deficit of plasma and red blood cell volumes. The plasma volumes ranged from 70 to 82 per cent on the expected normal, and red blood cell volumes from 54 to 80 per cent. When the lowered concentration of serum protein is also considered, the deficit of total circulating protein and especially albumin is even greater. The electrophoretic analyses (Table I) showed the usual nephrotic pattern with diminished albumin and γ-globulin and increased β-globulin in the serum. Colloid osmotic pressures were far below the normal range (Table II). The urine contained proportionately more albumin and less β-globulin than the serum.

Effects of injection of serum albumin

Plasma volume. The plasma volume rose sharply as the protein was injected (Figure 1). This was reflected both in the fall in dye concentration and in the lowered hematocrit determinations. Of the two, the hematocrit indicated the larger increase in plasma volume. This is notable, since the dye T-1824 is closely associated with the serum proteins and might be removed from the circulation with the albumin. There is no evidence for such an increased rate of disappearance.

Serum protein concentration. There was little immediate change in the concentration of pro-

TABLE II
Changes in plasma volume, circulating protein, and colloid osmotic pressure after injection of concentrated serum albumin

Patient	Time	Plasma volume	Serum protein	Total circulating Protein	Total circulating Albumin	Total circulating Globulin	Gain or loss from time of injection Albumin	Gain or loss from time of injection Globulin	Colloid osmotic pressure	Hematocrit
		cc.	*grams per 100 cc.*	*grams*	*grams*	*grams*	*grams*	*grams*		
I	Control	1800	3.40	61.2	8.0	53.2			100	35.2
	Calculated			86.2	33.0	53.2	+25.0			
	End of injection	2300	3.58	82.3						28.5
	30 minutes	2350	3.48	81.8	24.6	57.2	− 8.4	+ 4.0	117	26.9
	1 hour	2280	3.36	76.6					116	26.8
	6 hours	1920	3.51	67.5						32.0
	48 hours	1780	3.72	66.2	9.9	56.3	−23.1	+ 3.1	109	33.5
II	Control	2510	3.14	78.8	13.6	65.2			108	41.4
	Calculated			103.8	38.6	65.2	+25.0			
	20 minutes	3120	3.09	96.2					120	35.5
	40 minutes	3070	3.09	95.0					118	35.6
	1 hour	3080	3.00	92.4	22.1	70.3	−16.3	+ 5.3	118	35.4
	6 hours	3000	2.81	84.3						35.1
	24 hours	3080	2.50	77.0	15.4	61.6	−23.1	− 3.4	98	35.0
	48 hours	2980	2.26	67.2	12.5	54.7	−26.0	−11.3	86	35.9
III	Control	1820	3.70	67.3	10.1	57.2				48.2
	Calculated			92.3	35.1	57.2	+25			
	30 minutes	2340	3.70	86.6	25.3	61.3	− 9.8	+ 4.1		39.5
	24 hours	1910	3.75	71.6	13.6	58.0	−21.5	+ 0.8		43.8

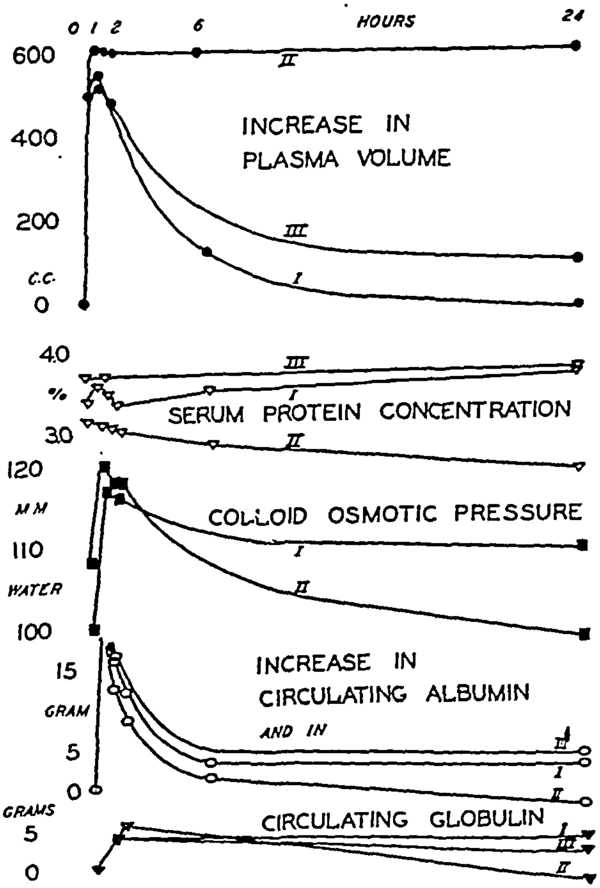

Fig. 1. Effect of Injection of Serum Albumin on Plasma Volume, Serum Protein Concentration, Colloid Osmotic Pressure, and Circulating Albumin and Globulin.

tein in the serum following a single injection of concentrated serum albumin. The concentrated protein solution must have been diluted or very rapidly removed from the circulation. In order to differentiate between these possibilities, it is necessary to calculate the total circulating serum protein.

Total circulating protein. When the serum protein concentration is multiplied by the plasma volume (expressed in appropriate units), the product represents grams of circulating serum protein. The product is far more informative than the individual factors, since a change in circulating protein may be reflected in variable changes in either concentration or plasma volume, depending on the exchange of fluid between the plasma and interstitial spaces in response to hydrostatic and osmotic forces. This is easily seen in Figure 1 in the contrast between case II on the one hand and cases I and III on the other. In all cases, there was a rapid fall in circulating protein after the injection, but the mechanism was not the same. In cases I and II, it was largely at the expense of the plasma volume, with the serum protein concentration stationary or rising slightly. In case II, however, the plasma volume maintained its increase at the expense of the protein concentration. This difference in mechanism, interesting in itself, must for the present only emphasize the more consistent behavior of the total circulating serum protein.

The results of the injection of albumin are presented in Table II. There is an increase of circulating protein after the injection, followed by a progressive decrease. Since the total circulating serum protein is the sum of the albumin and several globulin fractions, which may vary independently, the quantitative changes are considered under the individual fractions.

Albumin and globulins. The serum protein distribution, as determined by the Tiselius

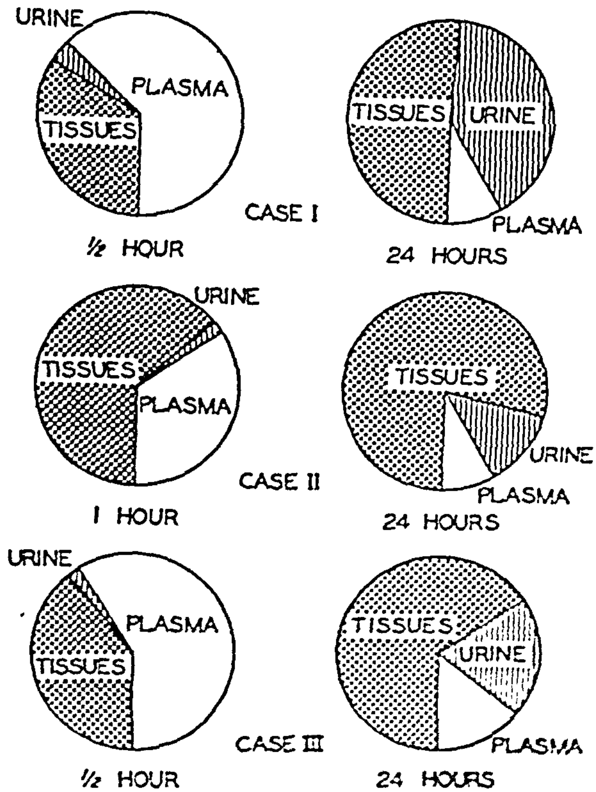

Fig. 2. Distribution of Injected Albumin between Plasma, Urine, and Tissues at Various Times after Injection

apparatus, changed far less than expected. When the total circulating serum albumin and globulins are calculated (concentration multiplied by plasma volume), it is evident that large and rapid transfers of protein have occurred. A large amount of albumin has apparently disappeared from the circulation and an appreciable amount of globulin has appeared within 30 minutes of the end of the injection. The amount of protein lost in the urine during the period involved in these transfers was insignificant in comparison.

In order to rule out some unusual error in the Tiselius determinations, the nephrotic serum was mixed with pure serum albumin *in vitro* and analyzed in a similar fashion. The results were very close to the expected values (Table V). This experiment would seem to eliminate any large analytical error and also any property of the serum of these patients which might affect the analysis.

Colloid osmotic pressure of serum. The colloid osmotic pressure increased following the injection, as might have been predicted from the increased proportion of albumin with little change in protein concentration.

Circulatory readjustments. There was a transient increase in arterial blood pressure during the injection. The venous pressure was increased by 10 to 30 mm. of water during the injection. This rise was sustained for several hours.

TABLE III

Effect of albumin injection on proteinuria

Case	Day	Protein in urine: Total	Albumin	α_1	Globulin	Protein clearance: Total	Albumin	α_1	Globulin
		grams per day				*cc. per minute*			
I	Control	11.2	5.2	2.3	3.7	0.23	0.82	0.65	0.10
	Injection	17.8	12.6	1.8	3.4	0.36	0.85	0.65	0.11
	2nd day	13.5	7.9	2.1	3.5				
	3rd day	12.6	7.0	2.0	3.6	0.23	0.85	0.56	0.09
II	Control	8.1	5.3	0.9	1.9	0.18	0.70	0.52	0.05
	Injection	10.4	8.3	0.7	1.4	0.24	0.80	0.66	0.05
	2nd day	8.1	6.1	0.7	1.3	0.22	0.84	0.46	0.05
	3rd day	7.3	4.9	0.9	1.5	0.22	0.80	0.68	0.06
	90-minute periods			*grams per hour* (α_1 + Globulin)				(α_1 + Globulin)	
III	Before	0.341	0.214	0.127		0.15	0.64	0.05	
	After	0.768	0.698	0.070		0.35	1.09	0.05	

In case III, α_1 could not be accurately measured in the serum because of its low concentration.

TABLE IV

Effect of albumin injection on glomerular filtration and on the excretion of chloride, protein, and T-1824

Clearences are expressed in cc. of plasma per minute per sq. meter of body surface.

	Mannitol	Chloride	Protein	T-1824	Albumin	Globulin
Before	101	0.35	0.09	0.37	0.39	0.030
After	177	0.25	0.21	0.75	0.66	0.030

TABLE V

Analysis of mixtures of albumin with nephrotic serum in vitro

	Protein	Albumin	
	mgm.	*per cent*	*mgm.*
SERUM A	108	8.8	9.5
Albumin	32	100.0	32.0
Mixture: calculated	140	29.6	41.5
observed		28.7	
SERUM B	119	17.9	21.3
Albumin	31	100.0	31.0
Mixture: calculated	150	34.9	52.3
observed		35.3	

Proteinuria. The loss of protein in the urine increased following the albumin injection. There was a rise both in the volume and in the protein concentration of the urine. The extra protein lost was entirely serum albumin, the daily globulin loss remaining nearly constant or diminishing somewhat (Table III). Within 2 days of the time of injection, proteinuria returned to the control level.

With a constant kidney status, the percentage of albumin in the urinary protein depends on the percentage of albumin in the serum protein, as suggested in a previous report (7). When the proportion of albumin in the serum was increased by the injection, the proportion of albumin in the urine became very large. With the subsequent fall in serum albumin, there was a fall in the proportion of albumin in the urinary protein (Figures 3, 4, and 5).

Albumin and globulin "clearance." The loss of protein in the urine may be compared with the concentration of protein in the blood plasma in the form of a clearance of plasma per minute. When this calculation is made on a daily basis, the albumin clearance and the globulin clearance

are little affected by the albumin injection (Table III). The clearance of α_1 is only slightly less than that of albumin and much greater than that of globulin. In case III, during the albumin injection, and for one hour afterward, the clearance of albumin was apparently increased; but because of the rapid shifts of protein, it is difficult to be certain of the mean serum albumin concentration. The clearance of total protein varies with the proportion of albumin in the serum, since the albumin clearance is much larger than the globulin clearance.

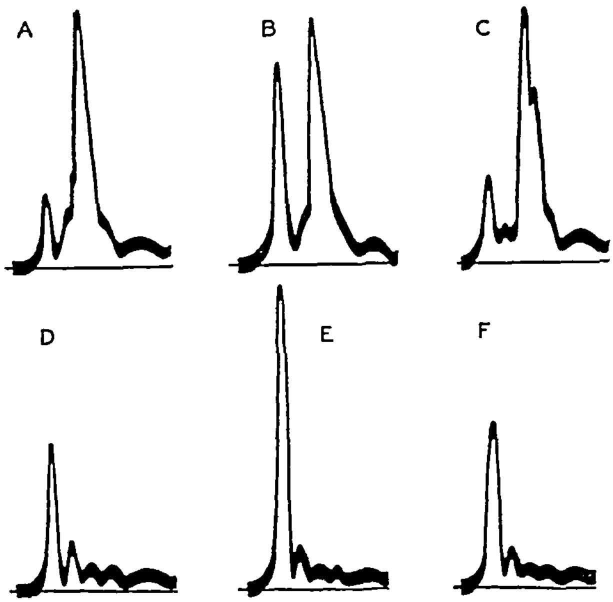

FIG. 3. ELECTROPHORETIC PATTERNS OF SERUM AND URINARY PROTEINS OF CASE I

A. control serum, B. serum ½ hour after injection, C. serum 48 hours after injection. Urinary protein patterns D, E, and F, correspond in time to the serum patterns above.

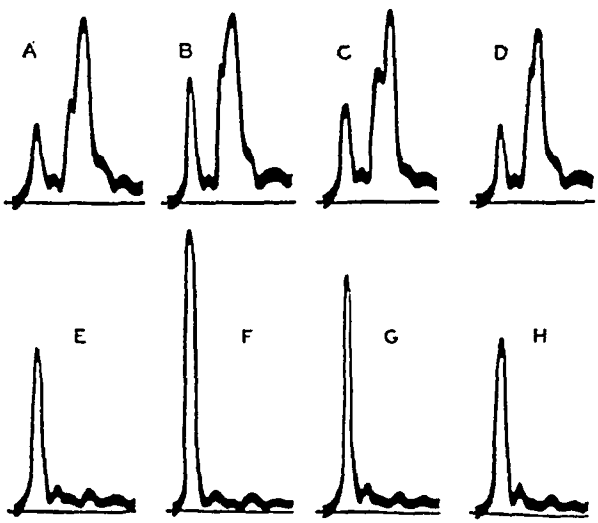

FIG. 4. ELECTROPHORETIC PATTERNS OF SERUM AND URINARY PROTEINS OF CASE II

A. control serum, B. serum 1 hour after injection, C. serum 24 hours after injection, D. serum 48 hours after injection. Urinary protein patterns E, F, G, and H correspond in time to the serum patterns above.

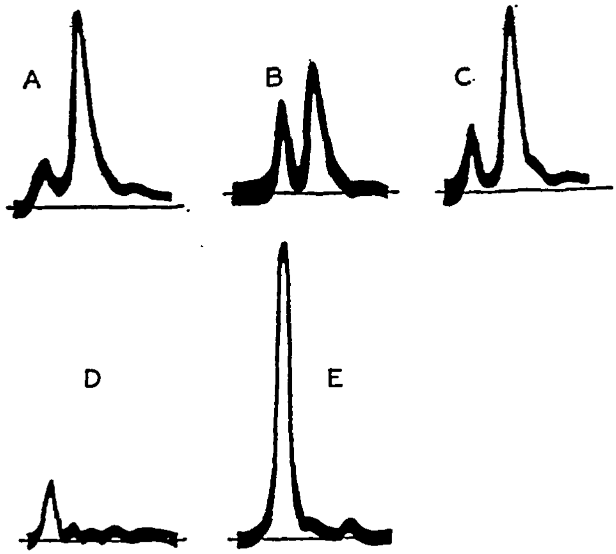

FIG. 5. ELECTROPHORETIC PATTERNS OF SERUM AND URINARY PROTEINS OF CASE III

A. control serum, B. serum ½ hour after injection, C. serum 24 hours after injection. Urinary protein patterns D and E correspond in time to the serum patterns above.

Clearance of the blue dye T-1824. The renal clearance of T-1824 from the plasma of these patients with nephrosis is proportional to the protein clearance and approximately four times as large, exceeding even the albumin clearance after injection of albumin.

Effect of albumin injection on fluid and salt excretion. Although a larger volume of urine appeared on the day of albumin injection, no increase in the daily excretion of sodium or chloride was noted. In case III, the mannitol clearance was increased following the injection of albumin, but the chloride clearance fell somewhat.

DISCUSSION

The magnitude of the osmotic forces set in motion by the injection of concentrated serum albumin, combined with the excess of interstitial fluid and the large deficit of blood volume,

readily explains the dilution of the blood observed. The failure of the serum protein concentration to rise is somewhat misleading, for the colloid osmotic pressure of the serum is significantly increased by the greater proportion of albumin.

The failure of the albumin-globulin ratio to rise to the calculated level was not unexpected, for the same observation has been made after the injection of plasma (2, 3). In both cases, albumin is rapidly withdrawn from the circulation. There is one important difference, however, in the direction of change in the circulating globulin. In the 3 cases receiving albumin, the circulating globulin increased following the injection, whereas there is a gradual decrease in circulating globulin following the administration of plasma. If the increase in globulin proves to be a constant finding, it suggests an available reserve of serum globulin which can be rapidly introduced into the circulation. All fractions, including α_1, are increased. The increased solubility of globulin in albumin solutions may play some rôle. The fluid with which the blood is diluted could scarcely contain the amounts of globulin involved. The conversion of albumin into globulin seems unlikely for many reasons, among which the most obvious are the rapidity of the exchange, the subsequent loss of globulin in the face of continued removal of albumin, and the maintenance of the same relative proportions of non-albumin fractions. In general, the observations suggest an equilibrium between circulating and reserve serum proteins, with a buffering of serum protein composition.

At the end of 1 or 2 days, only a small fraction of the injected albumin remained in the circulation. The loss of albumin in the urine was less than half of the quantity administered, and quite naturally varied inversely with the speed with which the albumin was removed from the plasma by the tissues, since these two routes of removal compete for a limited amount of albumin.

The 25 grams of serum albumin given to each of these patients represent 500 cc. of plasma or nearly a liter of blood. From the large proportion of the albumin removed from the circulation, it is evident that the equivalent of a number of liters of blood must be administered in order to produce clinical improvement, just as in the case of plasma.

Two major factors in the homeostasis of plasma protein concentration and distribution are evident in these experiments. The plasma volume can expand and contract to allow large changes in total circulating protein without significant change in protein concentration. The other stabilizing factor is the ability of the tissues to take up an excess of injected protein of one type and perhaps also to contribute small amounts of the other fractions, thus minimizing the change in the proportions of different fractions.

SUMMARY

The administration of a single injection of 25 grams of concentrated serum albumin was followed in 3 nephrotic patients by

(*1*) A rapid increase of plasma volume by 25 to 30 per cent of the original volume.

(*2*) A rise in the colloid osmotic pressure of the serum to 10 and 20 per cent above the original pressure (two patients).

(*3*) Small, variable changes in total serum protein concentration.

(*4*) An increase in circulating albumin which was always considerably less than the amount injected.

(*5*) A small but consistent increase in circulating globulin.

(*6*) An increased proteinuria due to the higher proportion of serum albumin with little change in albumin and globulin clearance, calculated on a daily basis.

(*7*) A small increase in urine volume but no rise in chloride excretion.

(*8*) No clinical benefit, inasmuch as the amounts given were far too small to produce any permanent change.

Within 48 hours after the injection, most of the effects had disappeared. The largest fraction of the injected protein had been removed from the circulation and was not to be found in the urine.

CONCLUSIONS

1. Further evidence has been presented for the equilibrium between circulating and tissue proteins.

2. As in the case of plasma, the equivalent in serum albumin of nearly a liter of normal blood fails to produce a persistent elevation of the circulating serum albumin in severe hypoproteinemia.

3. The deficit of red blood cell volume in patients with the nephrotic syndrome is again demonstrated. The concentration of red blood cells is further depressed when the plasma volume is increased by the administration of serum albumin.

4. The proteinuria in these patients could be related to the concentration of serum albumin and globulin by relatively constant renal clearances of albumin and globulin. The fraction designated α_1 resembles albumin in its clearance.

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SKIN TEMPERATURES OF THE EXTREMITIES OF PERSONS WITH INDUCED DEFICIENCIES OF THIAMINE, RIBOFLAVIN, AND OTHER COMPONENTS OF THE B COMPLEX

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Disturbances of vasomotor responses of the feet have been reported by Wenckebach (1), Weiss and Wilkins (2), and Wilkins and Kolb (3) among patients suffering with peripheral neuritis, associated with vitamin deficiency. The latter investigators stated that the disturbance was characterized by a decrease of the vasomotor tonus of the feet, resembling that found after resection of the first and second lumbar sympathetic ganglia. The vasoconstrictor response, as recorded by the plethysmograph, to brief vasoconstrictor stimuli was slight in the toes as compared with the fingers. Also, measurements of skin temperature showed that the toes were constantly warmer than the fingers; this was even more pronounced when the patients were subjected to a cool environment. After the body had been warmed, the skin temperature of the toes rose slightly or remained unchanged while the skin temperature of the fingers rose to or above that of the toes.

Since clinical and chemical studies of induced deficiency of thiamine, riboflavin, and other components of the B complex in human subjects were being carried out by Wilder, Mason, Cusick, Power and one of us (Williams) (4 to 6), the present studies were made in conjunction with these controlled studies to determine the amount of vasomotor disturbance in the extremities by means of measurements of skin temperature. Measurements of the loss of vasomotor tonus were made and correlated with the clinical signs and symptoms of deficiency disease, the apparent degree of depletion of the tissue stores of vitamins, and the severity of the metabolic defect.

METHODS OF STUDY

Selection of subjects. The studies were made on 8 physically healthy women from the nutrition division of the Rochester State Hospital. Their ages ranged from 25 to 48 years. During the period preliminary to restriction of the vitamins, the subjects were provided with an ample diet and daily supplements of 2.0 mgm. each of thiamine, of riboflavin, and of pyridoxine, 5.0 mgm. of calcium pantothenate, and 40.0 mgm. of nicotinamide for several weeks, so that a good status of nutrition could be assured prior to the restriction of the various vitamins.

The 8 subjects were divided into 4 groups of 2 subjects each and observations were made on (*1*) severe isolated restriction of thiamine hydrochloride, (*2*) the effects of thiamine deficiency on induced hyperthyroidism, (*3*) isolated restriction of riboflavin, and (*4*) restriction of the vitamin B complex.

Methods of observation. Physical and neurological examinations and electrocardiograms had been made and basal metabolic rates had been determined in the period of preliminary observation; these tests were repeated at intervals in the period of restriction of intake of vitamins and again in a subsequent period when adequate amounts of vitamins were provided. Criteria for determining the degree of depletion of vitamins were as follows: 1. *Determinations of tissue stores of vitamins.* We determined the "ordinary" excretions of thiamine and riboflavin in 24 hours, the subject receiving a measured amount of these vitamins in the diet. We also determined excretion of thiamine and riboflavin in the urine in 4 hours after subcutaneous injection of 1.0 mgm. of thiamine hydrochloride and 2.0 mgm. of sodium riboflavin, the subject being in the postabsorptive state. Excretions of thiamine and riboflavin are suggestive indexes of tissue store of these vitamins. 2. *Determination of biochemical status of tissues.* The levels of pyruvic acid and lactic acid in the blood after administration of dextrose are suggestive indices of biochemical status of tissue of the body. 3. *Clinical status of the subject.* Particular emphasis was placed on the development of objective signs of impairment of functions of the nervous system.

At various intervals, and more or less concomitantly with the other observations, the measurements of skin temperature were made in psychrometric rooms (7). The subjects had fasted for 15 hours prior to the tests and, during the time of testing, they wore light weight short pajamas and were in a supine position in comfortable beds. The temperatures of the plantar surfaces of the first and third toes of both feet and of the volar side of the distal phalanges

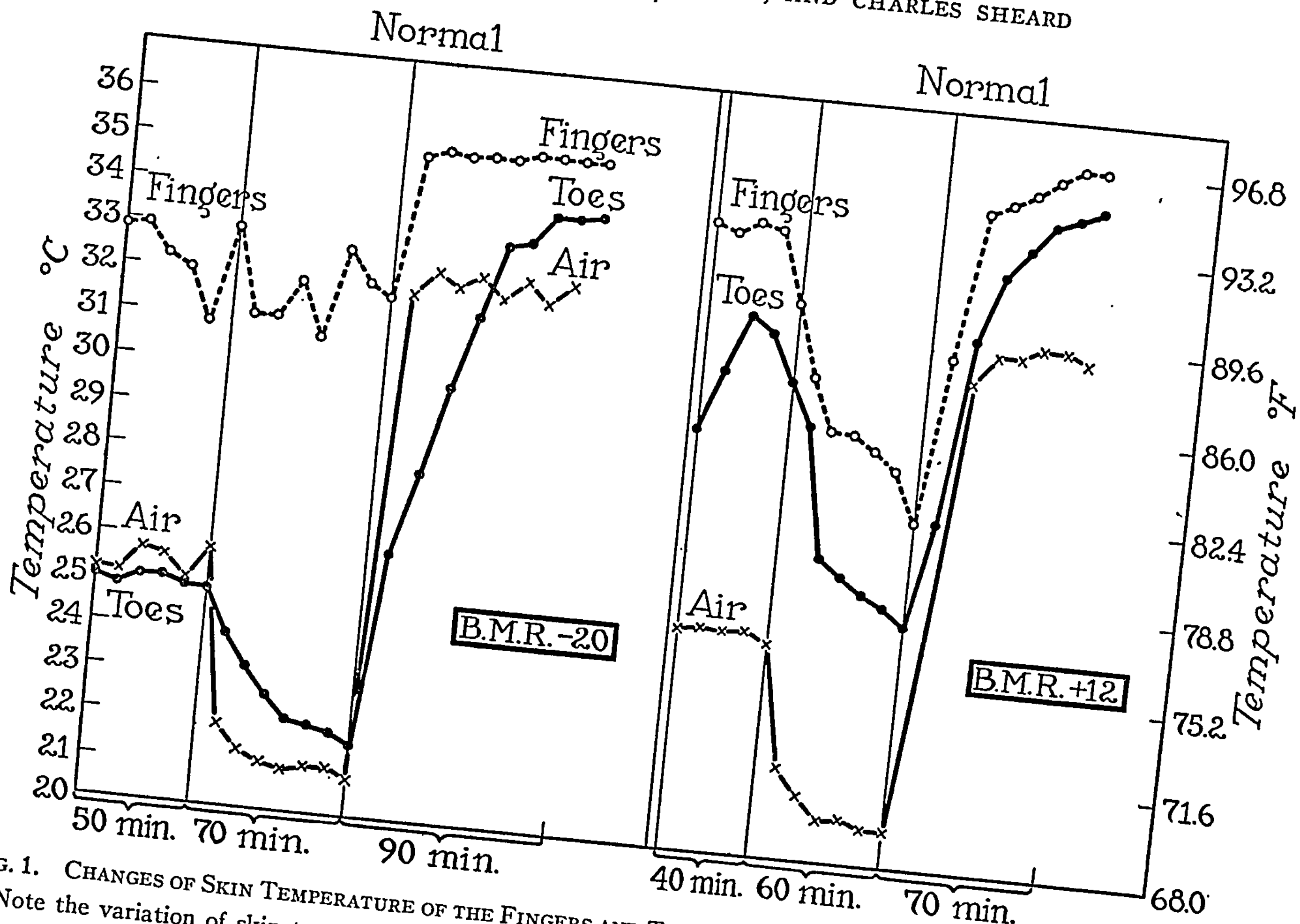

FIG. 1. CHANGES OF SKIN TEMPERATURE OF THE FINGERS AND TOES WITH CHANGES OF ENVIRONMENTAL TEMPERATURES

Note the variation of skin temperature of the fingers and toes associated with a variation of basal metabolic rate.

of the first and third fingers of the two hands were measured by means of copper-constantan thermocouples.

Since it had been shown that there is an approximately linear relationship (8) (which, however, seems to be of a dual character) existing between the average skin temperature of the toes and the basal metabolic rates obtained under environmental conditions of 25° C. (77° F.) with a relative humidity of 40 per cent, basal metabolic rates were determined on the previous day or the day after the measurements of skin temperature had been made.

Further, in order to demonstrate more clearly a loss of vasomotor tonus, skin temperatures were measured upon moving the subjects from a comfortable to a cooler environment and later to a warmer environment. Changes which occur in normal subjects under such conditions are demonstrated in Figure 1. When a normal person had remained for an hour or more at an environmental temperature of 25.5° C. (78° F.) with a relative humidity of 40 per cent, fairly constant readings were obtained and these showed a definite correlation with the basal metabolic rate. When the normal person was moved to a cooler environment of 20° C. (68° F.), there was relatively little thermal change in the forehead, thorax, arms, and upper portion of the legs. In contrast, there was definite cooling of the toes, and constancy of temperature (approximately that of the room) was reached in about an hour, except for those subjects who had higher basal metabolic rates.

The amount of cooling is closely associated with the basal metabolic rate. In turn, when the subject was moved from a room at 20° C. (68° F.) to one of 32° C. (89.6° F.), the changes of temperature were again most pronounced in the toes. The rise of temperature was rapid at first; this was followed by a gradual change as the temperature of the toes approximated a maximal value of about 35° C. (95° F.). The basal metabolic rate of the subject on the left (Figure 1) was −20 per cent, while that of the one on the right was +12 per cent.

In contrast, the loss of vasomotor tonus was demonstrated by observations following left lumbar sympathetic ganglionectomy and trunk resection, as is shown in Figure 2. The temperature of the toes on the left foot was approximately 35.0° C., which was 3° higher than the fingers, whereas the temperature of the toes on the right foot was approximately 27° C. When the subject was moved to an environmental temperature of 20° C. (68° F.), marked vasoconstriction occurred in the fingers and toes of the right side but the toes of the left foot remained warm. On moving him to an environmental temperature of 30° C. (86° F.), the temperature of the toes of the left foot was not appreciably changed while a marked rise of the surface temperature of the fingers and toes of the right side occurred. It was this kind of response that Wilkins and Kolb (3) found in a large number of their patients

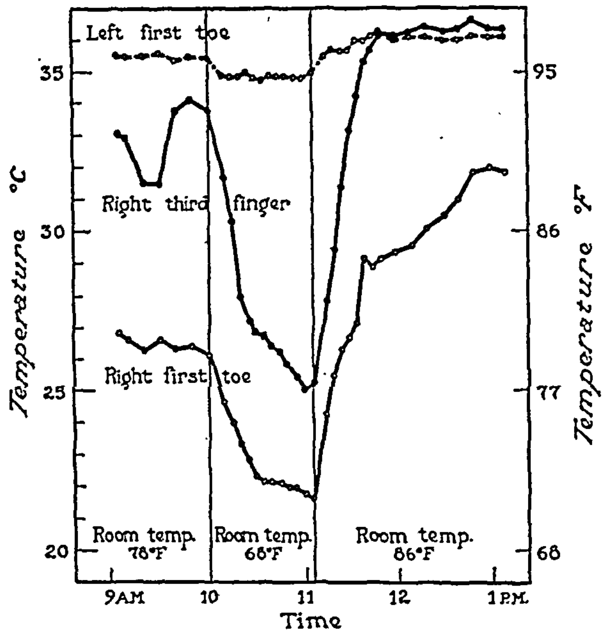

FIG. 2. CHANGES OF SKIN TEMPERATURE OF THE FINGERS AND TOES WITH CHANGES OF ENVIRONMENTAL TEMPERATURES FOLLOWING LEFT LUMBAR SYMPATHETIC GANGLIONECTOMY AND TRUNK RESECTION

(From Horton, Sheard and Roth: Vasomotor regulation of the temperatures of the extremities in health and disease. J. M. Soc. New Jersey [June] 1940).

ing with peripheral neuritis associated with vitamin deficiency.

RESULTS

Group I. Severe isolated restriction of thiamine (Table I). The intake of thiamine of 2 subjects, aged 48 and 36 years, was restricted to 0.175 mgm. per 1000 calories. Data on the excretions of thiamine, the concentration of pyruvic acid and lactic acid in the blood after administration of dextrose, and the neurologic status are contained in another report (4). Briefly, at the end of 110 days of restriction of vitamins, the severity of the thiamine deficiency was grade 3 on a basis of 1 to 4. The excretion of thiamine in the urine was decreased to a level of 10 to 20 micrograms for 24 hours. There was marked elevation of the concentration of pyruvic acid and lactic acid after the administration of dextrose. The amplitude of all the complexes of the electrocardiogram, particularly of the T waves, of CR_2 and IV R was somewhat decreased and sinus bradycardia and arrhythmia were present. Neurologically, the deep reflexes were decreased or absent (−3 to −4), muscle strength was impaired (−3), and the ability to arise from a squatting position was decreased, but the subjects could still walk. Paresthesia was present.

At the end of 120 days of restriction of thiamine, there was marked progression of signs and symptoms to grade 4. The excretion of thiamine was very low, the biochemical defect was severe, and a considerable degree of prostration was noted. The more severe neurologic defects occurred in subject 2, namely, paralysis of the *quadriceps femoris* and paresis of the other muscles of the legs. Perception of a wisp of cotton and pin point was diminished but not absent.

Following this period, subject 1 was given 0.22 mgm. of thiamine per 1000 calories daily for 58 days and then the dose was increased to 30 mgm. per 1000 calories for 37 days. At the end of this period, evidence of vitamin deficiency had disappeared except for a slight decrease of the deep reflexes. Subject 2 received 20 mgm. intravenously and 60 mgm. per 1000 calories, orally, for 31 days and then the daily intake was decreased to 15 mgm. per 1000 calories orally for 64 days. At the end of this time, weakness of the quadriceps femoris muscles was so great that she was unable to rise from a squatting position, and there was absence of the tendon reflexes of the legs. Figure 3 shows the measurements of skin temperature during the period of severest vitamin deficiency and after recovery from this deficiency. The curves of both subjects were practically the same; therefore, only one is presented. At the time when the thiamine deficiency was greatest, the basal metabolic rate was 0 per cent. Although there were a few transient changes of the basal metabolic rate during the period of recovery, at the time the measurements of skin temperature were made again, the basal metabolic rate was also 0 per cent. In both instances, vasoconstriction took place normally under the cool environment of 22° C. (71.6° F.) while vasodilatation followed on moving the subject to a hot environment of 32° C. (89.6° F.). This was indicated by a marked rise of the skin temperature of the toes. At no time were the toes warmer than the fingers.

Group II. Effects of thiamine deficiency on

TABLE I

Severe isolated thiamine deficiency and recovery

1. A woman aged 48 years, weight 55 kgm., height 161 cm.
2. A woman aged 36 years, weight 56.5 kgm., height 168 cm.

Number	Intake	Thiamine	Basal metabolic rate	Bio-chemical defect	Abnormal electro-cardiogram	Neurologic findings, grade: Deep reflexes	Neurologic findings, grade: Muscle strength	Neurologic findings, grade: Pares-thesia
	days	*mgm.*	*per cent*					
1	110	0.175*	−5	+3	+	−3 to −4	−3	+
	120	0.175*	0	+4	+	−3 to −4	−4	+
						Considerable prostration		
	Period of treatment							
	58	0.220*	−4	+2	0	−3 to −4	−1	0
	37	30.000*	0	0	0	−1 to −2	−1	0
2	110	0.175*	−7	+3	+	−3 to −4	−3	+
	120	0.175*	−10	+4	+	−4	−4	+
						Paralysis of quadriceps femoris; sensory perception, grade −2 to light touch and pain		
	Period of treatment							
	31	60 orally* 20 I. V.	+7	+2	0	−4	−4	+
						Inability to rise from squatting position; no sensory disturbance		
	64	15.000 orally*	−10		0	−4	−2	+
						Inability to rise from squatting position		

* Per 1,000 calories of daily diet.

induced hyperthyroidism. Since in earlier studies of moderate and prolonged deprivation of thiamine, in some instances, the basal metabolic rates of various subjects had been irregularly lowered and since a study was being carried out to determine the effectiveness of the thyroid hormone during periods of thiamine restriction, a second group of subjects was studied (Table II). The first of this group of subjects, who was aged 47 years, was given 0.6 grams (10 grains) of desiccated thyroid per day for a period of 185 days, and the second subject, aged 25 years, was given 0.5 grams (8 grains) of desiccated thyroid per day for the same period. The intake of thiamine was restricted to 0.45 mgm. per 1000 calories daily for a period of 108 days. Definite evidence of thiamine deficiency was present in both subjects at this time. The basal metabolic rate was increased to +19.1 per cent for the first subject and to +25.6 per cent for the second subject. Following this period, increasing amounts of thiamine were given over a period of 77 days. In spite of the increased intake of thiamine, definite evidence of vitamin deficiency was observed and was still present at the end of this time. In both subjects, there were muscle weakness and inability to rise from the squatting position. Apparently, the thyroid hormone was less effective for the maintenance of metabolic processes in states of thiamine deficiency than in the normal state, for concomitantly with the increase of intake of thiamine, the basal metabolic rates became higher. The skin temperature curves are shown in Figure 4.

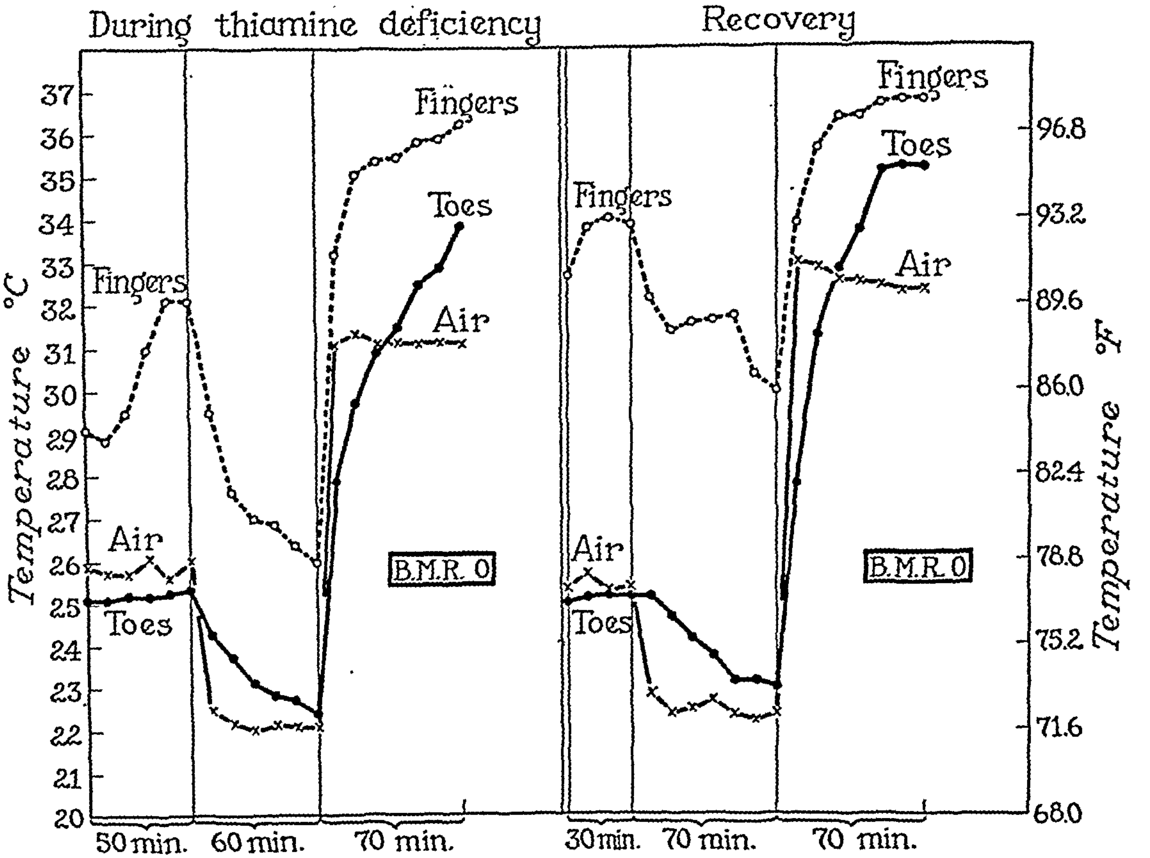

FIG. 3. A COMPARISON OF THE CHANGES OF SKIN TEMPERATURE OF THE FINGERS AND TOES WITH CHANGES OF ENVIRONMENTAL TEMPERATURES DURING THIAMINE DEFICIENCY AND FOLLOWING RECOVERY FROM THIS DEFICIENCY

Because the many skin temperature curves were similar, only one curve is shown. Again the correlation between the skin temperature of the toes and the basal metabolic rate was more evident than any correlation with known vitamin deficiency. In contrast, the skin temperatures of a patient who had hyperthyroidism without vitamin deficiency are shown. The basal metabolic rates were comparable and at no time did the skin temperature of the toes even approximate that of the fingers.

Group III. Isolated riboflavin deficiency. This group consisted of 2 subjects aged 42 and 32 years. They received an adequate diet except for riboflavin, which was restricted to 0.7 mgm. per day (0.35 mgm. per 1000 calories) over a period of 288 days. Data on the intake and excretions of riboflavin together with clinical observations have been reported (5). Briefly, the vitamin deficiency was demonstrated by unmistakable depletion of the tissue stores of riboflavin. The subjects had only occasional transient symptoms. The physical and neurologic examinations gave consistently negative results. The pyruvic acid and lactic acid levels were not elevated. The electrocardiograms and basal metabolic rates were unchanged. Ulceration, maceration, or excrescences at the corners of the mouth, at the nasolabial folds, beneath the pinna of the ears, or of any of the cutaneous folds were not observed. The tongue, the gums, and the vermilion border of the lips remained essentially normal. Measurements of skin temperature at various times during the period of restriction showed very little change. The skin temperatures of the toes showed more or less close correlation with the individual basal metabolic rates, which were little changed.

Group IV. Deficiency of the B complex. This group consisted of 2 subjects aged 44 and 30 years. The diet was deficient, so far as it could be judged, in vitamins of the B complex; it was

TABLE II

Isolated thiamine deficiency and induced hyperthyroidism

1. A woman aged 47 years, height 172 cm.
2. A woman aged 25 years, height 158 cm.

Number	Intake	Thiamine*	Desiccated thyroid	Basal metabolic rate	Biochemical defect	Abnormal electrocardiogram	Neurologic findings, grade	
							Deep reflexes	Muscle strength
	days	*mgm.*	*grams*	*per cent*				
1	103	0.45	0.6	+15.4	+4	+	−1 to −2	−2
	5	0.45	0.6	+19.1	+4	+	−1 to −2	−2
	19	0.75	0.6		+4	+	−1 to −2	−2
	17	1.07	0.6					
	15	1.55	0.6					
	15	1.65	0.6					
	11	15.00	0.6	+28.3	+4	+	−4	−3
	Total 185						Inability to rise from squatting position	
2	103	0.45	0.5	+24.3	+4	+	−1 to −2	−2
	5	0.45	0.5	+25.6	+4	+	−1 to −2	−2
	19	0.75	0.5					
	17	1.07	0.5					
	15	1.55	0.5					
	15	1.65	0.5					
	11	15.00	0.5	+28.0	+4	+	−4	−3
	Total 185						Inability to rise from squatting position	

* Per 1,000 calories of daily diet.

known to be deficient in thiamine and riboflavin. Restriction took place over a period of 243 days. Data on intake and excretions of thiamine and riboflavin are contained in a separate report (6). Signs and symptoms of thiamine deficiency were evident in 100 days. At the end of 243 days, the excretion of thiamine and riboflavin was decreased, the pyruvic acid and lactic acid levels in the blood were abnormally elevated following the administration of dextrose, but there were no objective neurologic changes present. The skin temperatures were more closely associated with moderate changes of basal metabolic rates than with the state of vitamin deficiency.

These observations are in contrast to those found by Wilkins and Kolb (3). Their studies for the most part were made on patients who had peripheral neuropathy, associated with alcoholism. The degree of depletion of tissue stores of vitamins and the severity of biochemical defects were not determined. Undoubtedly, the nutritional deficiencies of these patients had extended over a long period and irreversible changes may have occurred. In our studies, the degree and duration of restriction of vitamins were known, biochemical defects were demonstrated, and both neurologic and other physical defects were observed to develop with restrictions of vitamins and to disappear with administration of crystalline vitamins. Effects of ingestion of toxic substances and incidence of infectious processes were excluded by the conditions of the experiment.

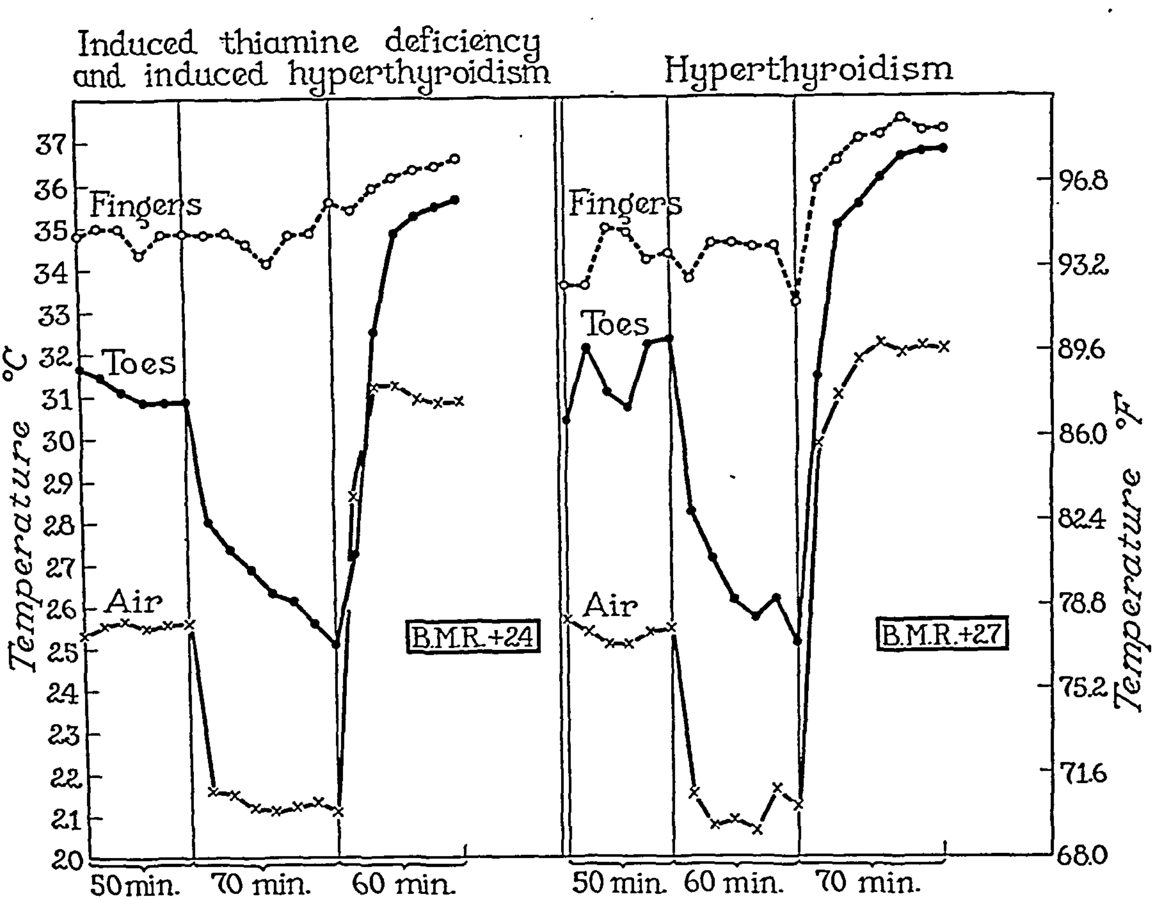

Fig. 4. A Comparison of the Changes of Skin Temperatures of the Fingers and Toes of a Subject with Changes of Environmental Temperatures During Induced Thiamine Deficiency and Induced Hyperthyroidism and One with Hyperthyroidism Only

SUMMARY

Under controlled environmental, postural, and metabolic conditions, none of the subjects of induced thiamine, riboflavin, or vitamin B complex deficiency showed any degree of vasomotor disturbance as evidenced by measurements of skin temperature and determination of rates of cooling and warming of body tissue.

When due consideration was given to the basal metabolic rate, the skin temperatures of the extremities under varying conditions were within the normal range. Furthermore, skin temperatures of the extremities of these subjects, measured at the height of the state of deficiency and following the administration of vitamins, showed a closer correlation with the basal metabolic rate than with the state of vitamin deficiency.

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THE RENAL CIRCULATION IN SHOCK[1]

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The status of the circulation through the kidneys during peripheral circulatory failure, or shock, is of interest inasmuch as kidney function is entirely dependent upon the circulation, and because a knowledge of the alterations in circulatory dynamics in one highly vascular region, such as the kidney, may contribute to the understanding of the mechanisms of regional redistribution of the circulation in the shock syndrome.

Clinical material. These studies formed an integral part of an investigation of shock in man, carried out at Bellevue Hospital. Methods of study and organizational detail have been previously reported (1). As far as possible, the procedures were planned to yield a comprehensive, well-integrated picture.

Renal clearances were measured in 35 patients, 18 to 71 years of age, of whom 27 were males and 8 were females.[3] They may be classified etiologically as follows: (*a*) skeletal trauma without shock, 3 cases; with shock, 11 cases; (*b*) hemorrhage without shock, one case; with shock, 9 cases (including 3 patients with cirrhosis of the liver and ruptured esophageal varices, and 3 who were later found to have mild or moderate essential hypertension); (*c*) peritoneal inflammation, 2 cases; one with necrosis of the lower colon and peritonitis resulting from a Lysol enema, but not in shock; and one in severe collapse from a strangulated hernia of 4 days' duration; (*d*) third degree burns, 2 cases; one in moderate shock and the other showing no evidence of shock; (*e*) head injury, 7 cases, all of whom were in coma and died shortly after study. Patients in group (*e*) were not in shock and stand in sharp contrast to the others. In 7 cases, complete studies were repeated 11 to 76 days after the episode of circulatory failure.

METHODS

The rate of glomerular filtration was measured by the clearance of either mannitol or inulin (both were used in a few cases) and the effective renal plasma flow was calculated from the clearance of sodium *p*-aminohippurate, except in a few instances where diodrast was used.[4] The plan of study was modelled after that of Smith, Goldring, and Chasis (5), but was freely modified according to the exigencies of each situation, which, from the nature of the investigation, were numerous. Analytical methods used are described in detail by Goldring and Chasis (6).

[4] Mannitol (25 per cent) and sodium p-aminohippurate (20 per cent) in ampoules were supplied through the courtesy of Sharpe and Dohme, Inc., and inulin (10 per cent) through the courtesy of the Warner Institute for Medical Research.

Dosage varied, but the following quantities were most often used: Priming: mannitol, 80 cc.; inulin, 20 to 30 cc.; p-aminohippurate, 9 to 13 cc. of 1.68 per cent solution. Infusion: mannitol, 3 to 4 per cent; inulin, 0.4 to 0.6 per cent, and p-aminohippurate, 0.2 to 0.24 per cent, where the rate of infusion was 4 cc. per minute. The infusion was made up in physiological saline obtained from the Sterisol Ampoule Company.

The clearance of p-aminohippurate has been found to be identical with that of diodrast in dogs (2) and in man (3). The identity of the clearance of inulin and mannitol in the *normal human* subject has been established (4). Simultaneous clearances performed in 4 cases of moderate or severe shock and 1 case of head injury (normal filtration rate and high urine flow in the last-mentioned) yielded an average mannitol/inulin ratio of about 0.90. *Since our* analytical method for inulin differed from that previously used in this laboratory, and since, at the time of comparisons, minor technical difficulties were experienced with the inulin method, it appears likely that the average difference of 10 per cent represents a systematic technical error rather than a true difference in clearance. For practical purposes, the clearances of mannitol and inulin in cases of shock are considered identical.

[1] This investigation was carried on under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Columbia University, with the collaboration of New York University; Dr. Dickinson W. Richards, Jr., responsible investigator, and Dr. Homer W. Smith, collaborating investigator.

[2] Now associated with the Department of Medicine, Boston University School of Medicine.

[3] Includes all patients studied up to March 1, 1943.

TECHNICAL SOURCES OF ERROR

Brief comment on some of the variables encountered will aid in proper evaluation of the data. The renal study was but one of several procedures carried out as nearly simultaneously as possible. Since all cases were emergencies, most of the planning had to be done rapidly. Cooperation varied greatly, being good with apathetic patients in severe shock and often poor with alcoholics. Washout of the bladder was sometimes made difficult by tensing of abdominal muscles or pain from adjacent injury. In the more severe cases, it was not always possible to carry out the renal study before starting blood transfusion, hence the results are sometimes complicated by the effect of treatment. Initial anuria occasionally made it necessary to postpone the study until after partial recovery. In order to obtain sufficient urine for analysis, collection periods were often longer than 15 minutes, although they seldom exceeded 30 minutes. Rapid changes in clearances might thus have been obscured. Because of the difficulty in predicting clearances, constant plasma concentrations were seldom obtained, but more frequent blood sampling minimized error caused by fluctuating levels. On the whole, it is believed that technical errors have been few and relatively small in magnitude. No case has been excluded from the data presented in this report.

RESULTS

Clinical data and results of the studies are summarized in Table I. Columns 9, 10, and 11 show the approximate values for cardiac output per minute per square meter of body surface (cardiac index), mean arterial pressure, and whole blood volume per square meter of body surface, all estimated to the midpoint of the renal study.[5] These are close approximations only (except blood pressures), but are useful in interpreting the results of the clearance studies.

The remaining columns of Table I present the clearance data and derived values corrected to 1.73 square meters of body surface. It should be emphasized that all values derived from the hippurate or diodrast clearances are understood to be qualified by the term "effective," as defined by Smith, Goldring, and Chasis (5). Effective whole blood flow was derived in the usual manner from the hippurate clearance and the hematocrit.

Columns 18 and 19 show two derived values which have proved useful in the study of renal hemodynamics. The effective renal vascular resistance is an over-all term derived from the mean arterial pressure and effective renal blood flow, and is defined as the loss of hydrostatic pressure between the renal artery and renal vein per unit of renal blood flow.[6] It is ex-

[5] This was done in one of two ways: If there had been no therapy prior to the renal study, and if the patient's condition was apparently stabilized, the actual hemodynamic measurements made before the kidney work (usually within the hour) were taken. If the conditions were changing, spontaneously or as a result of therapy, observations before and after the clearance study were utilized for the estimate.

[6] This concept derives from the law of Poiseuille, and may be applied to this situation as an approximation. If total resistance in the renal vessels is defined in terms of the loss of pressure from the renal artery to the renal vein (equivalent to loss of potential energy derived from the cardiac systole) and the blood flow through the kidneys, the only errors in the calculation will be those resulting from inadequate measurement of the terms of the equation itself, which is

$$R_K = \frac{\text{mean arterial pressure—renal vein pressure}}{\text{renal blood flow}}.$$

Mean pressure in the femoral artery is probably a good measure of the renal arterial mean pressure. Renal venous

Summary of renal clearance studies. Unless alcoholism was considered to dominate the clinical picture, its presence is not mentioned under etiology. Regarding treatment preceding the renal study, only significant therapy is noted, all patients having received varying quantities of physiological saline incidental to maintaining the patency of the intra-auricular catheter. The degree of shock existing when the group's studies were begun was evaluated as follows: 0, no shock; +, mild to moderate shock; ++, severe shock. For details of classification, reference is made to the previous report (1). By the time of the renal study, there was significant improvement in the condition of a number of the patients as a result of treatment, but because of the difficulty of estimating this improvement, only the original degree of shock is indicated.

In the majority of instances, 3 successive periods or less were measured. These have been averaged without regard to period-to-period variation, which was occasionally quite large. Where a study was repeated the results are presented separately. In 2 cases, G. J. and A. S., 7 and 6 successive periods, respectively, showed practically no variation, hence are presented as single averages. In 3 other cases, J. S., T. D., and M. M. (5, 9, and 7 consecutive periods), there were considerable changes, both in clearances and general hemodynamics, necessitating some arbitrary judgment in averaging periods for Table I and Figures 1 to 7. However, all periods are shown in the charts illustrating these cases (Figures 8 to 12).

The adrenal cortex extract, 50 Cartland-Nelson units per cc., was supplied through the courtesy of the Upjohn Company.

Sources from which the normal values for the hemodynamic and renal measurements were taken are given in the legends to Figures 1 to 7.

TABLE I

Summary of renal clearance studies

Patient	Age	Sex	S. A.	Etiological classification	Time of renal study after injury	Treatment preceding renal study	Initial degree of shock	Hemodynamic status at time of renal study: Cardiac index	Hemodynamic status at time of renal study: Mean arterial pressure	Hemodynamic status at time of renal study: Blood volume	No. of periods averaged	Urine flow	Filtration rate	Effective plasma flow	Effective blood flow	Filtration fraction	Effective renal vascular resistance	Effective renal fraction	Blood pH
			$M.^2$					*L. per minute per* $M.^2$	*mm. Hg*	*cc. per* $M.^2$		*cc. per minute*	*cc. per minute*	*cc. per minute*	*cc. per minute*	*per cent*	*A.U.*	*per cent*	
Normal			1.73					3.42	90	3000		Varies	131 ±22	669 ±128	1115 ±219	19.6	ca. 6500	ca. 19	7.42
W. H.	53	M	1.72	Trauma, alcoholism	6 hr.	None	0	3.68	104	3040	2	1.86*	***	1056	1965		4000	31.0	7.43
S. Ro.	20	M	1.91	Skeletal trauma	5 hr.	None	0	4.86	112	3300	1	6.28	162	837	1550	19.4	5700	18.4	7.40
J. S.	45	M	1.77	Trauma, alcoholism	5 hr.	100 cc. adrenal cortex	0	3.30	63	2675	4	0.58*	100	516**	823	19.4	6000	14.4	7.35
						mid-way	+	?	39	?	1	0.17*	39	173**	274	22.5	11000	?	
J. H.	56	M	1.81	Trauma, alcoholism	5 hr.	None	+	2.84	53	2300	2	1.02	24	635	931	3.8	4300	19.0	7.30
J. D.	62	M	1.77	Skeletal trauma	4 hr.	None	++	1.69	49	1780	2	2.64	72	539	857	13.4	4500	29.3	7.38
F. S.	61	M	1.99	Skeletal and chest trauma	6 hr.	None	+	2.70	54	2420	3	2.53	70	432	758	16.3	4900	16.1	7.41
P. Y.	55	M	1.79	Skeletal trauma	6 hr.	800 cc. blood	++	?	82	2100	3	1.94	54	236	369	22.8	17000	?	7.41
G. J.	63	M	1.56	Skeletal trauma	5 hr.	None	+	2.40	66	2120	7	0.86	24	137	180	17.5	29000	4.1	7.39
T. D.	48	M	1.64	Trauma, alcoholism	6 hr.	None	+	3.60	64	2900	2	0.23*	8	107	175	7.5	29000	2.9	7.31
					7 hr.	During 500 cc. blood		3.60	75		2	0.57*	28	427	701	6.5	8500	11.5	
					8 hr.	After 500 cc. blood		3.60	86	3100	4	0.80*	61	480	794	13.5	8700	12.8	7.32
J. V.	49	M	1.80	Skeletal trauma	8 hr.	None	++	1.30	39	1640			Too low to be measured						
					13 hr.	1450 cc. blood		3.16	77	2200	2	2.30	59	167	256	36.2	23000	4.7	7.22
A. A.†	53	F	1.68	Skeletal trauma	10 hr.	500 cc. blood	++	3.00	54	2000	3	0.13	3	23	33	13.0	137000	0.6	7.30
V. B.	65	M	1.55	Skeletal and chest trauma	13 hr.	None	++	1.85	53	2240	2	0.41	10	53	75	19.2	63000	2.4	7.34
S. R.	60	M	1.75	Skeletal trauma	3 hr.	None	++	2.19	45	1660			Too low to be measured						7.22
					8 hr.	2000 cc. blood		<3.20	67	1790	3	0.83*	21	124**	215	16.9	25000	ca. 4.0	7.25
					20 hr.	500 cc. blood		2.50	71	1960	2	0.57*	13	53**	84	24.5	67000	2.4	7.32
M. M.	48	F	1.47	Skeletal trauma	5 hr.	100 cc. adr. cortex	++	1.87	51	1610	2	0.35*	35	238	340	14.7	14000	10.8	7.33
					7 hr.	During 1500 cc. blood		3.96	85	2000	5	2.06	81	381	531	21.2	15000	13.3	7.42
R. W.	42	M	1.91	Gastric hemorrhage	24 hr.	None	0	2.69	91	1760	2	2.26	112	454	669	24.5	9700	14.3	7.44
C. F.	64	F	1.58	Knife wound hemorrhage	15 hr.	850 cc. blood	++	1.96	85	1700	2	2.95	47	213	336	22.1	22000	10.0	7.38
H. M.†	60	F	1.58	Intestinal hemorrhage	12 hr.	600 cc. blood	++	2.24	45	2200	2	0.34	6	118	175	5.1	22000	4.5	7.37
P. P.	53	M	1.86	Gun-shot wound of abdomen,	10 hr.	None	++	0.67	20	2100			Complete anuria						6.90
				hemorrhage, refrigeration	21 hr.	2000 cc. blood-$NaHCO_3$		<3.70	50	<3100	2	0.22*	7	55	95	12.7	41000	ca. 1–2	7.20
A. S.	60	F	1.51	Gastric hemorrhage—mild	48 hr.	During 1000 cc. blood	++	2.40	92	1850	6	0.52*	55	287	374	19.1	22000	9.5	7.48
			1.54	ess. hypertension	+3 da.	None		3.60	64	1700	2	1.31*	88	373	490	23.4	12000	7.9	7.47
M. Mc.	54	F	1.54	Ext. hemorrhage, alcoholism,	7 hr.	None	+	1.84	50	1680			Too low to be measured						7.31
				mild ess. hypertension	10 hr.	1500 cc. saline, rapid		3.90	78	>1600	2	2.83*	69	157	217	44.0	34000	<3.2	7.24
E. C.	40	M	1.69	Gastric hemorrhage, ess. hy-	4 hr.	None	+	2.36	97	2290	3	0.65	15	113	148	13.3	53000	3.6	7.34
				pertension	11 hr.	None		2.48	99	2110	3	0.41	7	103	126	6.8	64000	2.9	7.41
M. S.	49	M	1.59	Cirrhosis, ascites, hemorrhage	5 hr.	None	+	4.25	38	2490	2	0.91	39	491	595	7.8	5500	8.8	7.40
A. H.	63	M	1.70	Cirrhosis, hematemesis	17 hr.	None	++	1.55	44	1500			Too low to be measured						7.22
					20 hr.	1000 cc. blood		2.10	66	1850	4	0.72	9	101	140	8.9	38000	3.8	7.09
S. M.	51	M	1.79	Cirrhosis, hematemesis	?	500 cc. blood	+	3.72	52	?			Too low to be measured						7.01

TABLE I—*Continued*

Patient	Age	Sex	S. A.	Etiological classification	Time of renal study after injury	Treatment preceding renal study	Initial degree of shock	Hemodynamic status at time of renal study: Cardiac index	Hemodynamic status at time of renal study: Mean arterial pressure	Hemodynamic status at time of renal study: Blood volume	No. of periods averaged	Urine flow	Filtration rate	Effective plasma flow	Effective blood flow	Filtration fraction	Effective renal vascular resistance	Effective renal fraction	Blood pH
			*M.*²					*L. per minute per M.*²	*mm. Hg*	*cc. per M.*²		*cc. per minute*	*cc. per minute*	*cc. per minute*	*cc. per minute*	*per cent*	*A.U.*	*per cent*	
L. P.‡	21	F	1.59	Necrosis of colon, peritonitis	24 hr.	None	0	4.53	72	2110	3	5.61	120	747	1464	16.5	4300	18.8	7.42
J. Do.‡	71	M	1.72	Intestinal obstruction	4 da.	None	++	2.26	73	2620	2	0.17	2	11	22	18.2	265000	0.6	7.46
J. R.‡	18	M	1.84	3° burns, face, hands	16 hr.	300 cc. plasma	0	2.06	104	2150	3	1.85	***	574**	1173		6700	32.8	7.46
R. G.	46	F	1.38	3° burns, extensive area	9 hr.	900 cc. plasma	+	1.81	?	2300	3	0.67*	123#	332**	615	36.9		19.8	
A. C.‡	62	M	1.76	Head injury	6 hr.	None	Coma	4.26	105	?	3	4.10	74	796	1375	9.5	6000	18.6	7.52
S. B.‡	40	M	1.65	Head injury	5 hr.	None	Coma	4.23	94	3000	2	6.80	198	980	1605	20.2	4900	22.0	7.51
C. LeC.	46	M	1.89	Head injury	12 hr.	None	Coma	4.37	94	2370	3	1.90*	167	616	998	27.1	6800	13.3	7.53
J. He.	49	M	1.63	Head injury	5 hr.	None	Coma	3.43	98	2850	3	3.64	134	657	1153	20.4	7200	19.4	7.41
C. L.‡	24	M	1.74	Head injury	5 hr.	None	Coma	3.16	68	2550	3	2.96	104	479	851	21.7	6300	15.6	7.37
S. Mi.‡	21	M	2.14	Head injury	7 hr.	None	Coma	2.20	78	3070	3	6.66	107	416	751	25.4	6700	19.7	7.45
H. F.‡	54	M	1.62	Head injury	?	None	Coma	3.41	90	2600	3	2.89	49	328	587	14.9	13100	9.9	7.48
J. S.	45	M	1.74	Recovered, normal	32 da.	None		3.92	110	3190	3	3.07	130	795**	1432	16.3	6100	21.2	7.45
L. P.	21	F	1.48	Recovered, normal	38 da.	None		5.34	116	2430	3	4.52	125	866	1332	14.7	8100	14.4	7.43
M. M.	48	F	1.42	Recovered, normal	25 da.	None		4.25	100	2200	3	1.43*	116	771	1266	15.0	7700	17.2	7.40
R. W.	42	M	1.92	Recovered, normal	17 da.	None		3.81	97	2560	2	2.68	109	690	1096	15.7	6400	16.6	7.42
J. D.	62	M	1.61	Recovered, normal	45 da.	None		3.45	94	2580	3	2.43	123	587	890	20.9	9100	15.4	7.42
T. D.	48	M	1.61	Recovered, normal	76 da.	None		3.92	108	2950	3	2.44	66	582	887	11.3	10000	14.9	7.44
M. Mc.	54	F	1.55	Recovered, hypertensive	11 da.	None		3.92	133	2110	3	2.29*	84	423	605	19.8	19000	6.9	7.55

* Inulin used for filtration rate.
** Diodrast used for plasma flow.
† Pyrogenic reaction from infusion (A. A. Temp. 107°; H. M. 103.6°).
‡ Fever present over 101° (R.).
# Technically not entirely satisfactory—May be up to 20 per cent too high.
*** Inulin clearance discarded because plasma level was too low and hence too near to the blank value of the blood to be accurate.

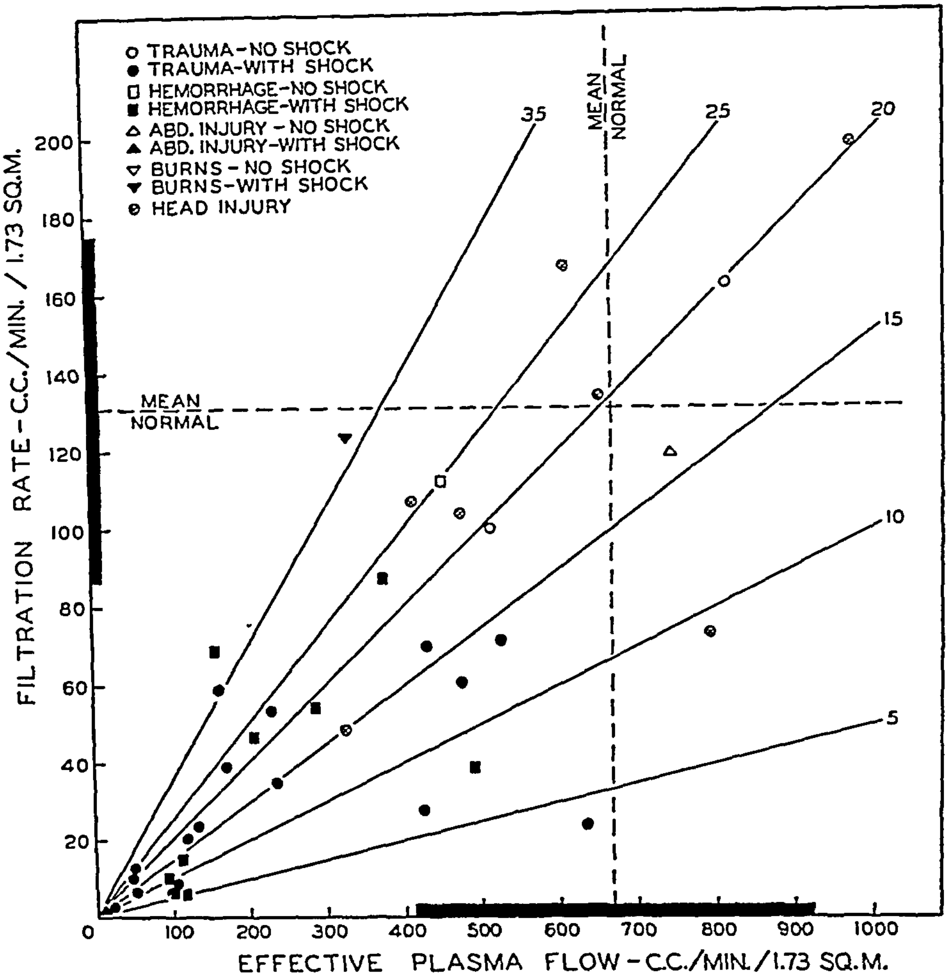

FIG. 1. SHOWING RELATIONSHIP BETWEEN FILTRATION RATE AND RENAL PLASMA FLOW

In this and succeeding charts, each point represents the average of several clearance periods, and all cases given in Table I are shown. The solid columns on the ordinate and abscissa represent ± two times the standard deviations for normal males and females, taken from Goldring, Chasis, Ranges, and Smith (8). The diagonal lines define 5 per cent intervals of filtration fraction. E.g., all points along the line, "20," represent a filtration fraction of 20 per cent. Note that the majority of values from patients in shock (solid symbols) fall below the normal ranges of both filtration rate and plasma flow. The tendency toward high filtration fractions, in spite of pyrexia, in the head injury cases is clearly shown.

pressed in absolute units as dynes cm.$^{-5}$ second. Renal fraction expresses the proportion of the total cardiac output which perfuses the functional renal tissue, and is given by the ratio:

$$\text{renal fraction} = \frac{\text{renal blood flow (cc. per minute)} \times 100}{\text{cardiac output (cc. per minute)}}.$$

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pressure is certainly small in comparison to P_m, but since it has not been measured in intact man, the term has arbitrarily been set equal to zero. The third factor, renal blood flow, derives from the plasma clearance of hippurate or diodrast and is subject to the same limitations as the clearance itself. In practice, the mean pressure is converted to dynes per cm.^{2} by the factor of 1332, the blood flow is expressed in cc. per second, and the result given in absolute units.

It will be noted that the definition does not indicate how or where the hydrostatic energy is dissipated, but only how much. Whether in a given case the greatest resistance is afferent or efferent to the glomerulus is not given by our calculation. Lamport (7) has derived equations which attempt to determine this distribution, but we are not yet prepared to apply them to our data. However, the overall resistance value seems more useful for examining the altered relationship between the renal and systemic circulations in shock.

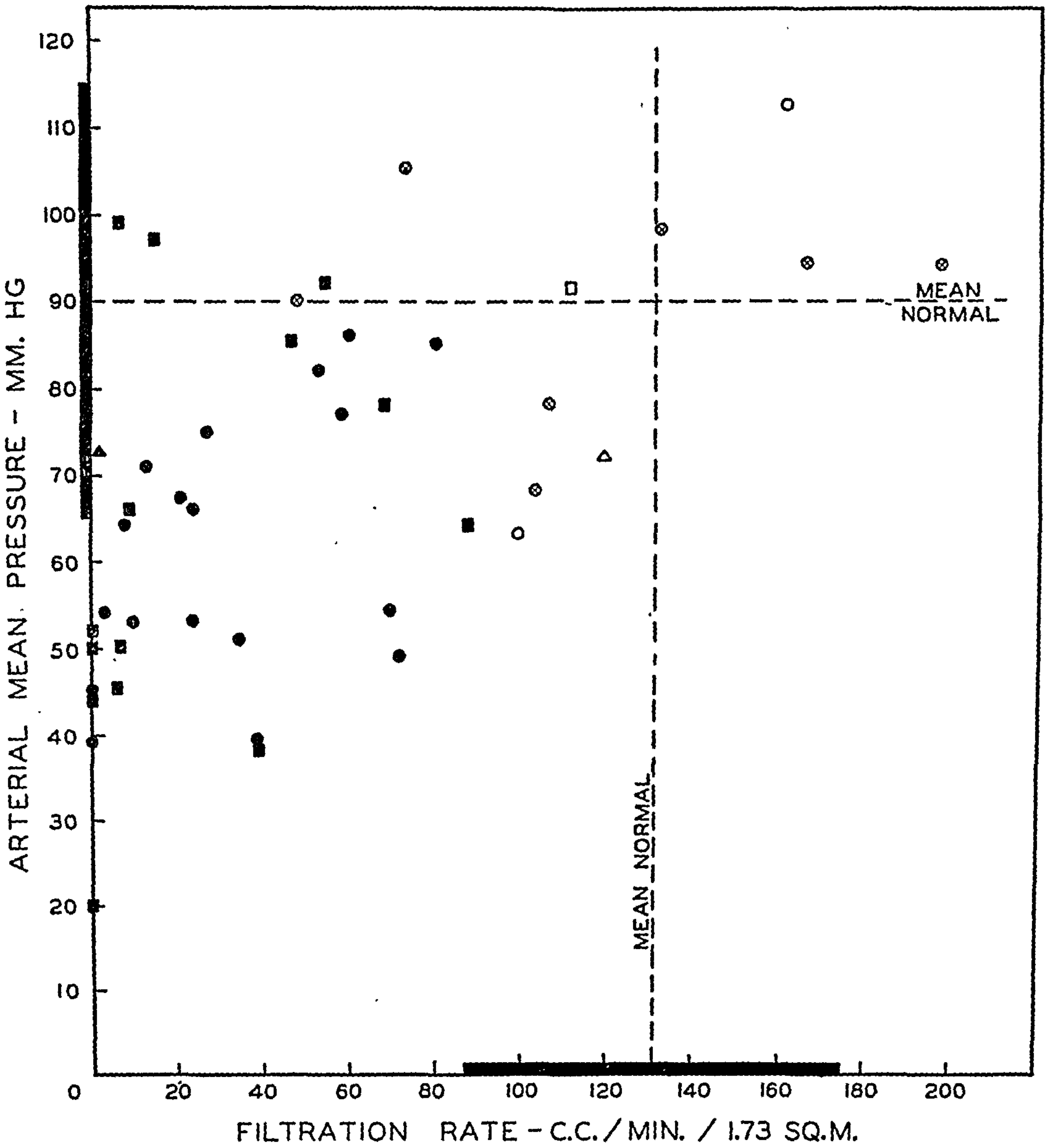

FIG. 2. RELATIONSHIP BETWEEN MEAN ARTERIAL BLOOD PRESSURE AND RATE OF GLOMERULAR FILTRATION

The normal range for the latter is the same as in Figure 1. Mean arterial pressure was obtained by planimetric integration of femoral pressure pulse tracings recorded by means of a Hamilton type manometer (9). The normal range for mean pressure was modified from data of Bradley and Smith (10). Note that in most shock cases, the decrease in filtration rate is greater than the corresponding fall in arterial mean pressure.

A decrease in the renal fraction, simultaneous with a decrease in total cardiac output, indicates that functional tissue in the kidneys is receiving less than its normal *proportion* of the blood flow; and conversely, that other areas outside the kidney, or non-functioning tissue in the kidney, must be receiving more than the usual proportion. In other words, change in this value indicates the shunting of blood to or away from the functional renal tissue relative to the normal condition. The effective renal vascular resistance is chiefly of value in determining whether a reduction in renal blood flow is the result solely of decreased arterial pressure or whether additional factors are present.

The major results of this study may be summarized in the statement that the rate of glomerular filtration and effective renal plasma flow are significantly reduced in nearly every patient suffering from shock, the degree of reduction being roughly proportional to the severity of shock. The data of Table I are

shown graphically in Figures 1 to 7, which are presented to illustrate the chief alterations in renal vascular dynamics.

1. *Filtration rate, plasma flow, and filtration fraction.* The values for filtration rate and plasma flow range from a high normal level in patients who suffered injury or hemorrhage but who were not in shock, down to practically zero in the most severely shocked patients. The scatter of data may be seen in Figure 1, in which filtration rate is plotted against plasma flow. All the data from patients not in shock lie within the normal range. In the head injury group, the clearances were normal, or slightly above or below the normal range. The diagonal lines define intervals of filtration fraction. The

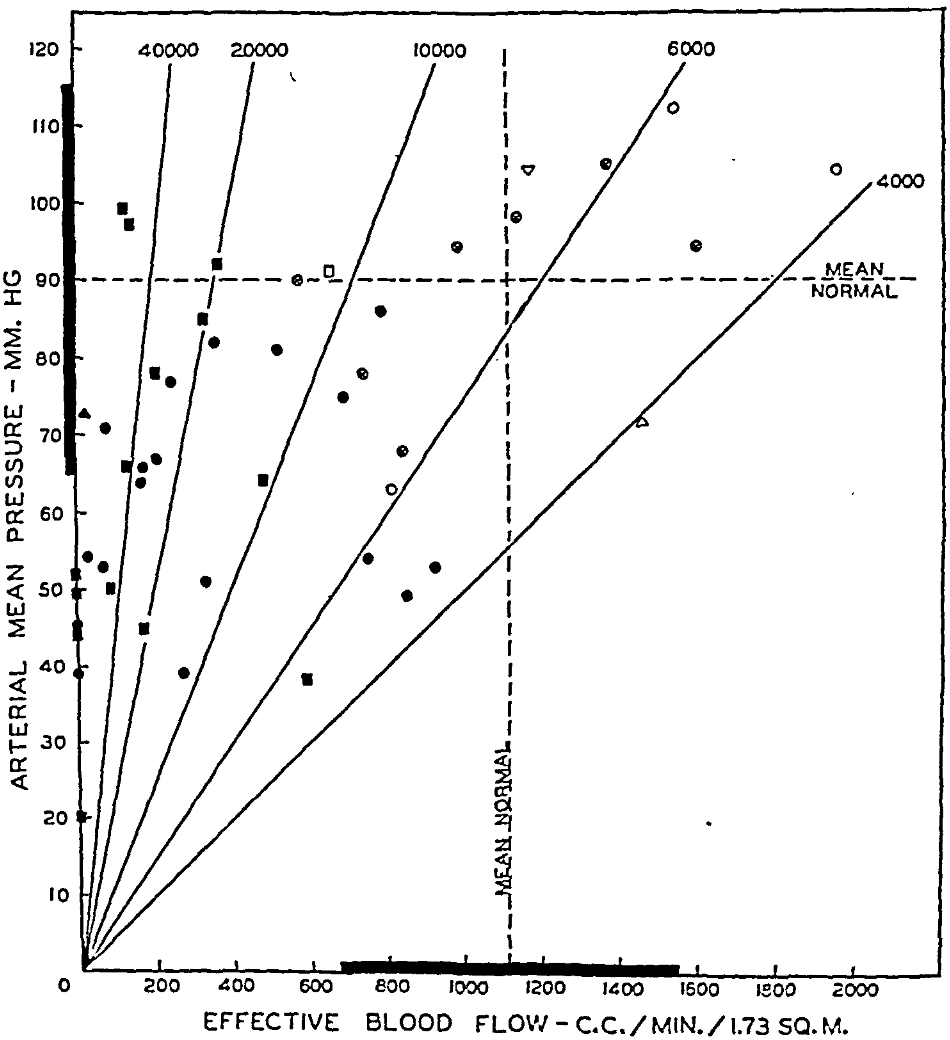

FIG. 3. RELATIONSHIP BETWEEN ARTERIAL MEAN PRESSURE AND RENAL BLOOD FLOW

The normal range for the latter was taken from Goldring, Chasis, Ranges, and Smith (8). The diagonal lines originating from zero indicate successive values, expressed in absolute units, for effective renal vascular resistance. Thus, all points along a given line have the same value for resistance, the magnitude of which expresses the ratio

$$R_E = \frac{P_m}{\text{R.B.F.}}, \text{ converted to absolute units.}$$

The normal range is approximately 4000 to 10,000 A. U. Note that in most shock cases, the points lie in zones of increased resistance, indicating that vasoconstriction occurred in the kidneys. See text for details.

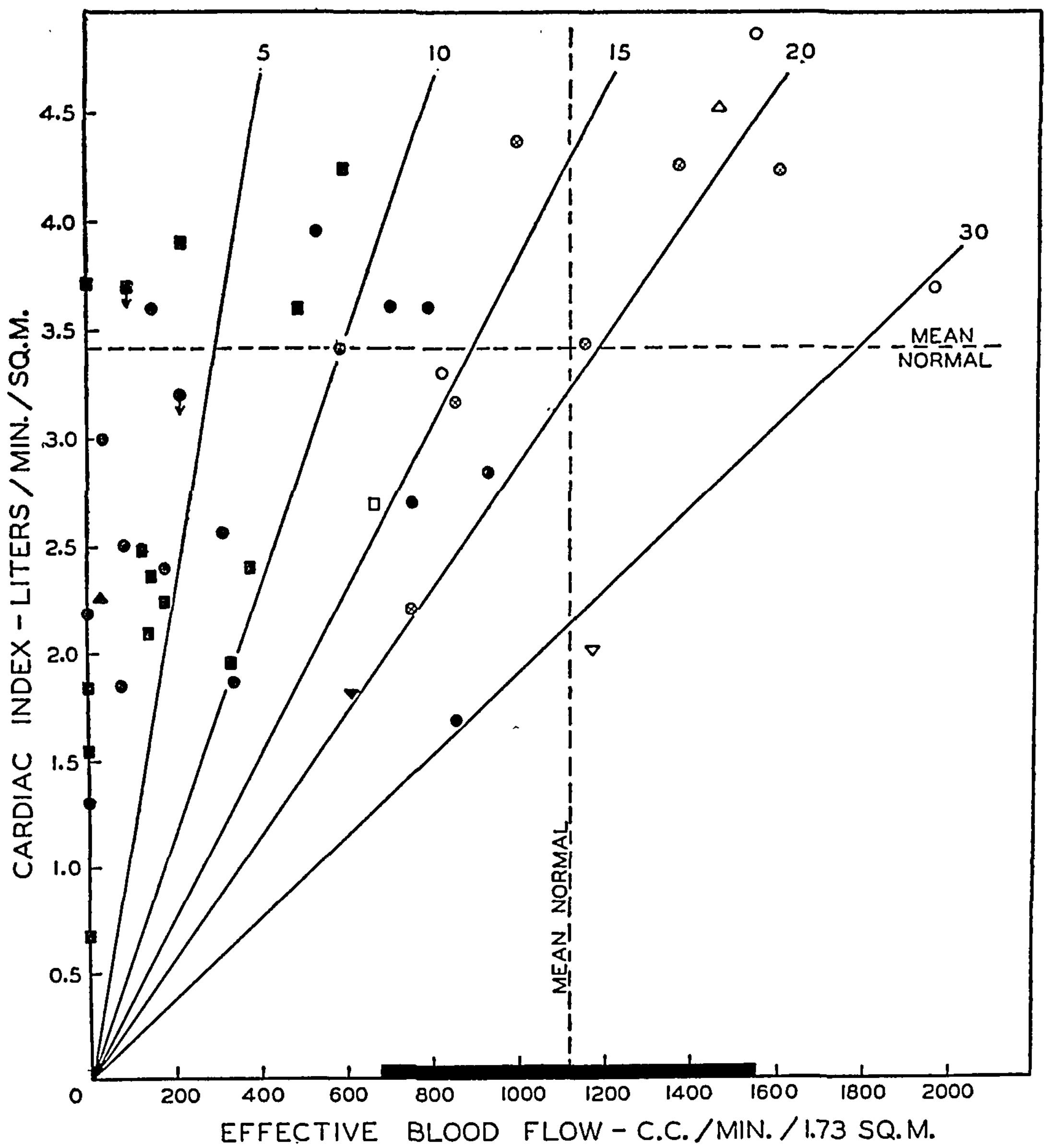

FIG. 4. RELATIONSHIP BETWEEN CARDIAC OUTPUT IN LITERS PER MINUTE PER SQUARE METER OF BODY SURFACE AND THE RENAL BLOOD FLOW IN CC. PER 1.73 SQUARE METERS

Cardiac output was measured by the Fick method, for details of which see Cournand *et al.* (1). The diagonal lines originating from zero indicate successive values of the renal fraction, expressed as per cent, calculated on the basis of the "ideal" surface area of 1.73 square meters. This arbitrary choice was necessary since the renal fraction expresses the ratio of renal blood flow to the *total* cardiac output rather than to cardiac output per square meter of surface area. For surface areas larger or smaller than 1.73 square meters, the corresponding renal fraction lines would lie closely adjacent to the lines shown in this chart. Hence, the latter are mathematically accurate only for the surface area of 1.73 square meters, and are intended solely as approximate guides for the evaluation of the relationship between cardiac output and renal blood flow.

In the normal recumbent man, about 15 to 25 per cent of the cardiac output flows through the kidneys. It is seen that in most shock cases, the renal fraction was considerably less than normal, indicating that as the cardiac output decreased, blood was shunted away from the kidneys. The two points with inverted arrows represent clearances obtained fairly long after the last cardiac output determination, and it is likely that by the time of the renal study, the cardiac output had decreased somewhat.

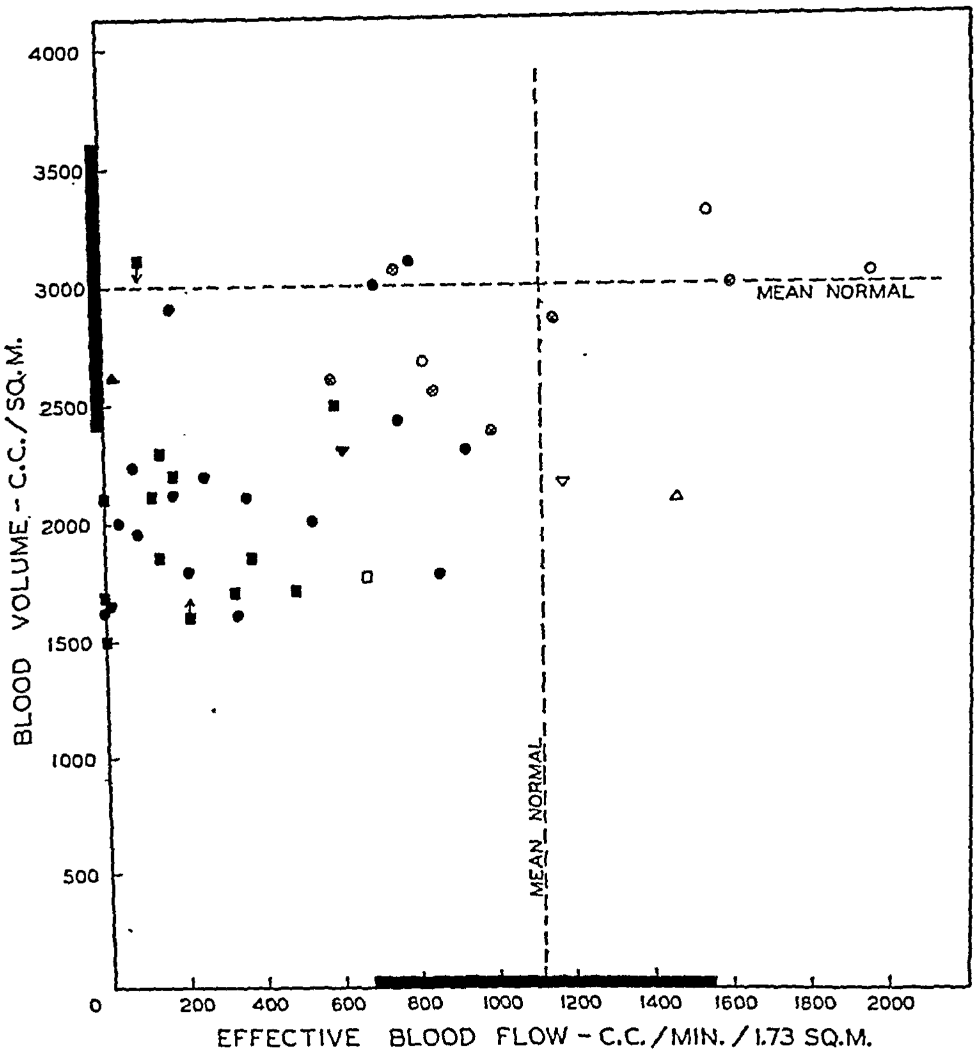

FIG. 5. CORRELATION BETWEEN BLOOD VOLUME IN CC. PER SQUARE METER OF BODY SURFACE AND THE RENAL BLOOD FLOW

The normal range of blood volume is an approximation based on data gathered from the literature and from their own experience by Drs. M. I. Gregersen and R. P. Noble. Blood volume was determined by the dye method of Gregersen, for details of which see Cournand *et al.* (1).

The correlation is improved somewhat by the deletion of the three points in the upper left of the chart. In P. P. (indicated by the inverted arrow), the last blood volume measurement was made more than 3 hours before the clearance study, and since the patient's blood pressure and general condition had deteriorated during that time, it is probable that further blood loss had occurred. Case J. Do., represented by the solid triangle, had suffered from strangulated hernia for 4 days, making it likely that the toxic and infectious factors contributed to his collapse in addition to loss of body water. Furthermore, it is probable that the hippurate clearance underestimates the true renal blood flow if there has been prolonged renal anoxia which could reduce the extraction ratio. The third symbol represents the initial value of T. D. in whom severe acute alcoholism was believed to have caused marked fall in blood pressure in spite of a cardiac output and blood volume near normal (see Figure 10).

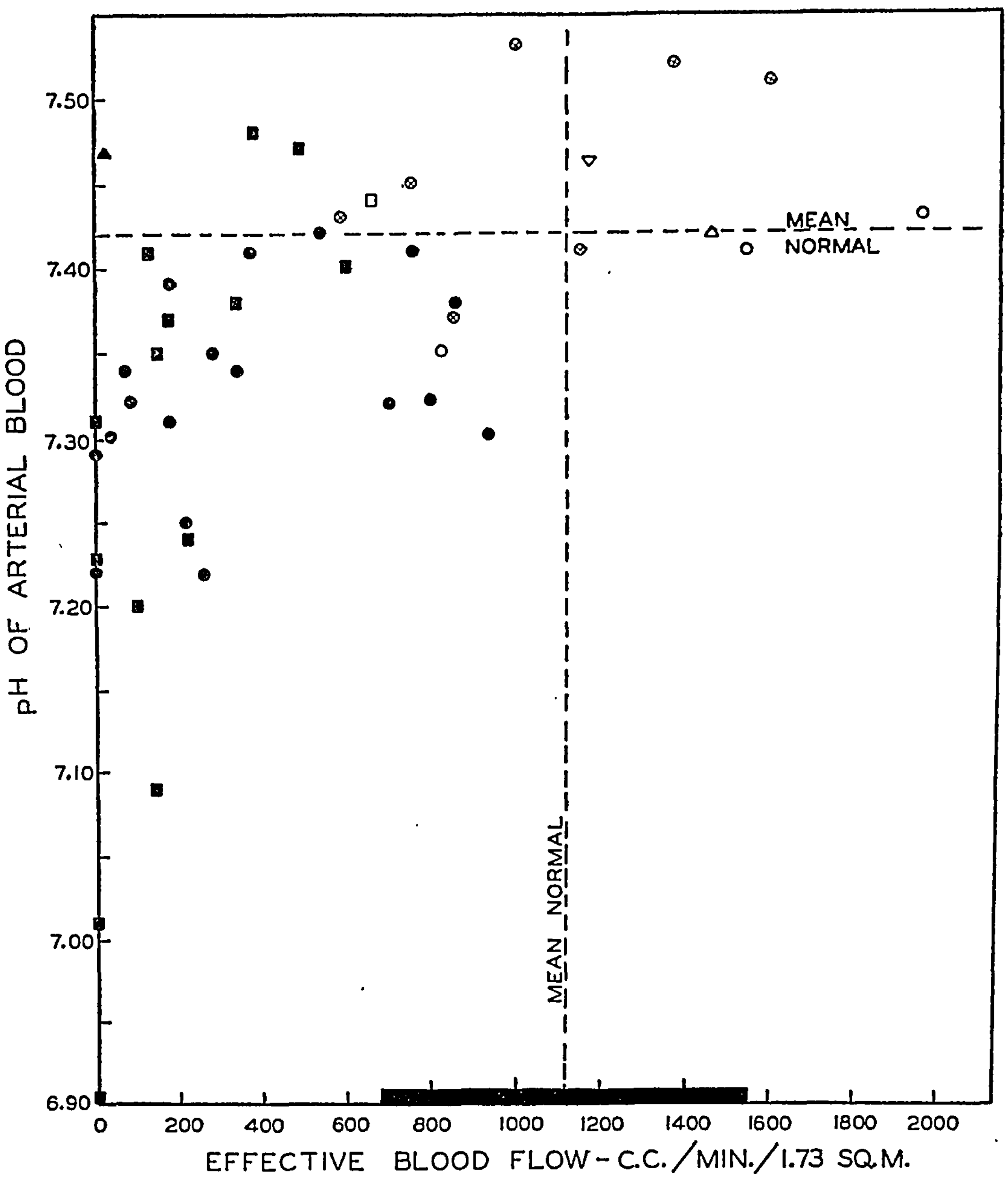

FIG. 6. RELATIONSHIP BETWEEN THE pH OF ARTERIAL BLOOD AND THE RENAL BLOOD FLOW

See Cournand *et al.* (1) for method of measurement of pH. Note that some extremely low blood flows were associated with normal blood pH, a fact which precludes the possibility that acidosis is the primary cause of renal ischemia in shock. See discussion in text.

individual points tend to be grouped almost equally above and below the average normal filtration fraction of about 20 per cent.

Table II is an analysis of the data shown in Figure 1, in terms of several variables encountered in the study. The range of 15 to 25 per cent approximately defines the normal limits of filtration fraction. The tendency toward higher filtration fractions is clearly seen in 5 of the 7 head injury cases, a fact worthy of emphasis, since the degree of fever seen in most of these cases would ordinarily be associated with a reduced filtration fraction. The 4 patients classified as not being in shock fall in the normal range, whereas the filtration fraction was below normal in 13 of the shock cases and above normal in 2.

2. *Filtration rate related to arterial blood pressure.* Figure 2 shows in general that the rate of filtration decreased with fall in mean arterial pressure, a result that would be anticipated from the fact that the glomerulus functions as a passive filter. In most cases, however, the filtration rate fell far more than could be ac-

TABLE II

Analysis of Figure 1

		Filtration fraction			Total
		<15	15 to 25	>25	
Total values in Figure 1		14	20	4	38
Head injury		1	4	2	7
No shock		0	4	0	4
Shock		13	12	2	27
Excluding head injury cases	Predominantly alcoholic	4	2	0	6
	Predominantly non-alcoholic	9*	14	2	25
	No treatment	8	9	0	17
	Treatment with blood or plasma	6*	6	2	14

Excludes all values from Table I which are designated as "too low to be measured." W. H. and J. R. not shown because filtration rate is lacking. Also excluded is M. Mc. because treatment differed from the others.

* Two of these patients suffered severe chills and fever at the time of renal study (A. A. and H. M.). Hence it is conceivable that their filtration fractions might have been higher in the absence of pyrogenic reactions.

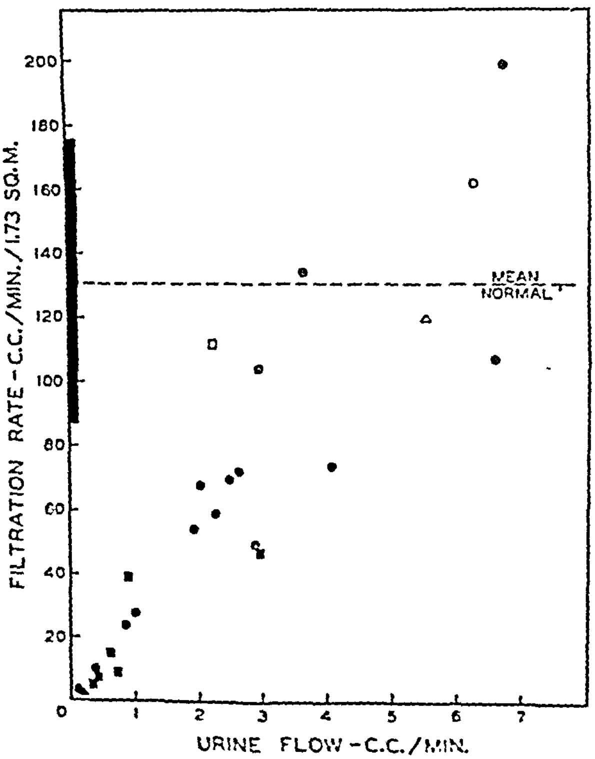

FIG. 7. CORRELATION BETWEEN RATE OF FILTRATION AND URINE FLOW IN ALL CASES IN WHICH MANNITOL WAS USED TO MEASURE THE FILTRATION RATE

It is to be emphasized that this high degree of correlation is a consequence of the osmotic diuretic action of the mannitol. See text for details.

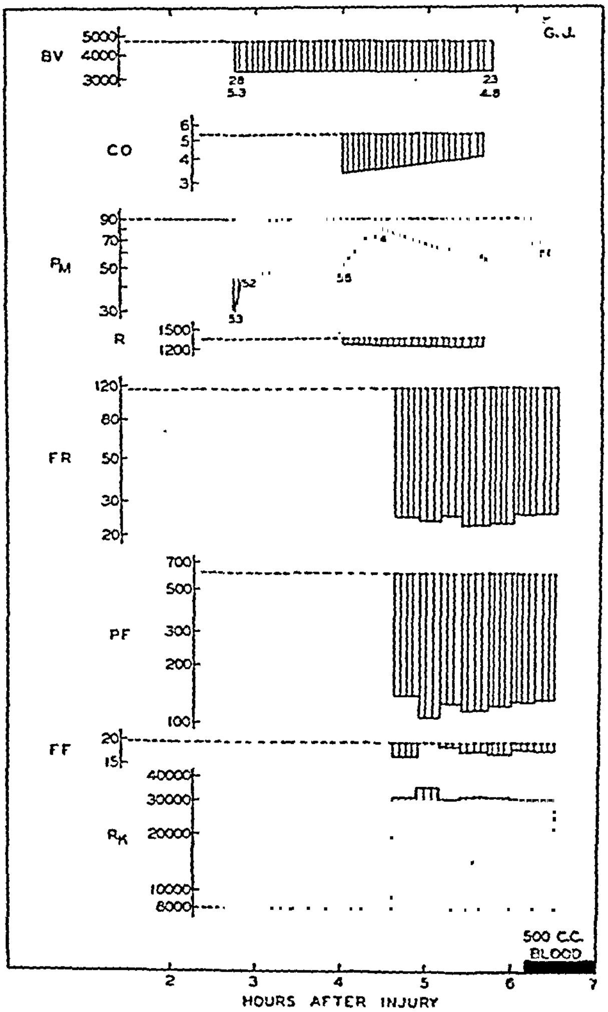

FIG. 8. G. J., 65-YEAR-OLD MALE. COMPOUND FRACTURES OF EXTREMITIES AND RUPTURED SPLEEN. USUAL SIGNS OF SHOCK PRESENT

Normal averages are used as base-lines since no control study was done. All ordinates are plotted logarithmically. *BV*, total blood volume in cc.; *CO*, cardiac output in liters per minute; P_M, mean arterial blood pressure in mm. of mercury; *R*, total peripheral resistance in dynes $cm.^{-5}$ seconds (absolute units); *FR*, rate of glomerular filtration in cc. per minute; *PF*, effective renal plasma flow in cc. per minute; *FF*, filtration fraction in per cent; R_K, effective renal vascular resistance in dynes $cm.^{-5}$ seconds. The small figures below the blood volume represent hematocrit and plasma protein values, while those beneath blood pressure indicate pulse rate.

Blood pressure rose from initial low level of 30 mm. of mercury and ranged from 82 to 62 during the renal study. The stability, at a depressed level, of both general and renal circulations is well shown in this case. The urine flow averaged only 0.05 cc. per minute during the first hour, but increased to about 1.0 cc. per minute after mannitol was given for the renal study (osmotic diuresis). Blood transfusion was begun just before the study was terminated to permit surgical treatment of the fractures. The bradycardia was probably related to bundle branch block discovered in an electrocardiogram.

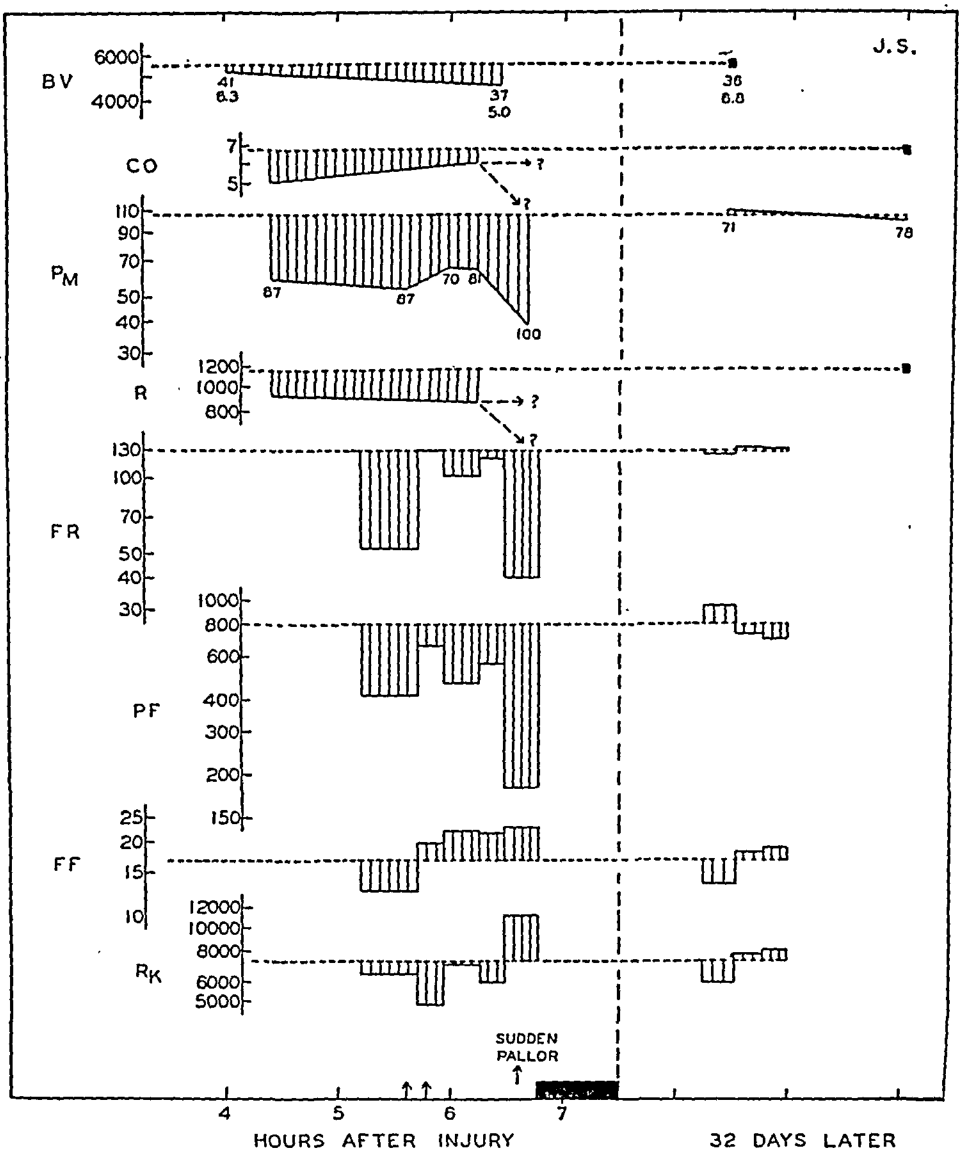

FIG. 9. J. S., 45-YEAR-OLD MALE. MULTIPLE COMPOUND AND COMMINUTED FRACTURES OF BOTH BONES OF RIGHT LOWER LEG, AND ACUTE ALCOHOLISM

Units of measurement as in Figure 8. Base-lines indicate values obtained in a repeated study made 32 days after injury.

Other than a moderate decrease in blood pressure, there was no evidence of shock until about 2 hours after the beginning of the study, at which time (just before transfer to the operating room), the skin became pale and clammy, the blood pressure dropped sharply, and the renal blood flow and filtration rate decreased almost proportionately. Renal clearances were not greatly depressed prior to the fall in blood pressure. Adrenal cortex extract (Upjohn), 100 cc. intravenously, given at the times indicated by the arrows, had no definite effect on general hemodynamics and the apparent rise in clearances following the injections is not different from that observed spontaneously in other cases in which no cortical extract was given (see Figure 10). The variability of the clearances indicates a vasomotor instability which contrasts with the steady state seen in Figure 8. Whether the sudden collapse was due primarily to arteriolar dilatation, or to failure of venous return and decreased cardiac output (venous pooling), cannot be answered since cardiac output was not measured at the time. Alcoholism is believed responsible for most of the initial hypotension (decreased peripheral resistance). Note the progressive hemodilution. The solid block on the time scale at the end of original study indicates transfusion of 500 cc. of whole blood.

counted for by the degree of systemic blood pressure reduction. Most of the patients had hemodilution, which decreases the physical viscosity and the colloid osmotic pressure of the blood. Reductions in each would tend to result in a greater filtration for any given level of blood pressure than was observed. It is probable, therefore, that there was an actual increase in the afferent arteriolar resistance in most cases. The variation found was considerable, suggesting that the several factors varied in their relative importance. For example, filtration rates between 10 and 20 cc. per minute were associated with mean arterial pressures ranging from 50 to 100 mm. mercury. Conversely, a mean arterial pressure of 70 mm. mercury produced a rate of filtration of only 3 cc. per minute in one case and 120 cc. per minute in another.[7]

3. *Renal blood flow as related to arterial blood pressure; renal resistance.* The outstanding feature in Figure 3 is the striking reduction in effective renal blood flow observed in the shock syndrome. The diagonal lines originating from zero define various levels of renal vascular resistance, and were computed directly from the equation

$$R_K = \frac{P_m \times 1332}{\text{R.B.F. per second}}.$$

If the flow of blood through the kidneys decreased in proportion to the reduction in blood pressure, the regression of R_K would occur within the normal resistance range of 4000 to 10,000 absolute units. That such is not the case, however, is evident from the fact that most of the values representing patients in shock are found in zones of increased resistance. Hence, it may be stated that the reduction in renal blood flow was greater than the corresponding fall in blood pressure because of increased resistance in the kidneys. Since the hematocrit was reduced in most instances, a circumstance which, by itself, would tend to decrease resistance, it follows that a considerable degree of renal vasoconstriction must have been present in most of the cases.

4. *Renal blood flow and cardiac output; renal fraction.* The relationship between cardiac output (total systemic blood flow) and the renal blood flow is shown in Figure 4. In this chart, the diagonal lines indicate increasing values of the effective renal fraction, calculated from the ratio

$$\text{renal fraction} = \frac{\text{R.B.F.}}{\text{C.O.}}.$$

In only one case where shock was present (J. D.), was the renal fraction greater than the average normal of about 19 per cent. The reduction in effective renal blood flow was greater than the decrease in cardiac output in the majority of shock cases, hence the renal fraction was decreased, indicating that renal vasoconstriction shunted blood away from the functional tissue of the kidneys, presumably to other parts of the body.

5. *Renal blood flow as related to blood volume.* *A priori*, a correlation between renal blood flow and the circulating blood volume would be expected on the basis of the oligemia theory of shock. Figure 5 was prepared with this point in mind. Although there is less correlation between decreasing blood volume and decreasing renal blood flow than was seen between blood pressure and cardiac output and the renal blood flow, it will be noted that most of the values representing patients in shock fall below the normal ranges of both blood volume and renal blood flow.

6. *Renal blood flow and pH of the blood.* The development of uncompensated acidosis during shock suggests a possible relationship to renal

[7] Quantitative evaluation of the factors involved in glomerular dynamics is difficult in normal subjects (7, 11), but becomes more so in an abnormal state like shock. Decrease in the glomerular capillary permeability is unlikely. Furthermore, it is probable that pressure in Bowman's capsule opposing filtration is not increased, but actually may be decreased. Thus, a low filtration rate associated with a fairly high arterial pressure can only mean marked increase in frictional resistance in the afferent vessels.

The results in Case M. S. were unusual in that the relatively high filtration rate of 39 cc. per minute was observed when the mean arterial pressure was at the very low level of 38 mm. mercury. Since the plasma protein and hematocrit were reduced to 4.4 grams and 22 per cent, respectively, it is believed that the factors of viscosity and osmotic pressure reduction played a large part, although afferent vasodilatation may have been present in addition. The filtration fraction was only 7.8 per cent, a value which would ordinarily imply efferent dilatation (11). In reality, this case is too complex to be so simply explained, and serves to illustrate the difficulties of quantitating glomerular dynamics under highly abnormal circumstances.

function. Although Figure 6 shows some correlation between decrease of blood pH and reduction of renal blood flow, there are some important exceptions to the general trend, e.g., marked reduction in renal blood flow in the presence of normal blood pH. Moreover, in several cases during treatment, the renal blood flow increased while the blood pH continued to fall (see J. V., M. Mc., and A. H. in Table I). It has been observed that the renal circulation is apparently reduced promptly following injury or hemorrhage, whereas organic acid accumulation depends upon the duration as well as the degree of the circulatory failure (12).

7. *Urine flow in shock.* Oliguria was observed in nearly all of the shocked patients in whom the urine flow was measured. In some of the most severe cases, complete anuria occurred, and not until the general circulation had improved as a result of treatment did urine flow resume. It is probable that anuria in shock actually indicates almost complete cessation of renal circulation, since the lowest measurable blood flow values were only 5 to 10 per cent of normal, under conditions where corresponding urine flows were still measurable.

The great difference in molecular weight between inulin and mannitol (5000 : 181) and the fact that the urinary concentrations were very different (about 5 to 10 times greater in the case of mannitol, in our study) accounts for the fact that the use of mannitol was accompanied by larger urine flows. Thus, most of the values for urine flow shown in Table I are considerably higher than would have been observed if mannitol had not been given. This is clearly shown in Table III. Where mannitol was used, the U/P ratio ranged from 9 to 53, with a mean of 27. With inulin, the range was 19 to 243, averaging 84. With both inulin and mannitol, the U/P ratio was lowest when the filtration rate was less than 20 cc. per minute (averaging 20 for mannitol and 36 for inulin), suggesting some derangement of the concentrating function in the most severe cases. This is in accord with the observation that the specific gravity of the urine in shock tends to become fixed at about 1.013 (13), and with our own observations in a few cases, that the urine in severe shock tends to become isotonic with plasma, in spite of marked oliguria. It is interesting to note that in no case was the reabsorption of water less than 89 per cent of the quantity filtered, and in most cases, even in the presence of mannitol, more than 93 per cent was reabsorbed.

TABLE III

Showing the osmotic diuretic effect of mannitol
Includes all periods from all cases.

Range of filtration rate	Mannitol		Inulin	
	Number of periods averaged	Urine flow	Number of periods averaged	Urine flow
cc. per minute		*cc. per minute*		*cc. per minute*
1 to 20	20	0.4	6	0.3
21 to 40	9	1.0	5	0.6
41 to 60	6	2.3	9	0.5
61 to 80	13	2.5	7	1.7
81 to 100	7	3.5	5	1.5
101 to 120	8	3.5		
120 and up	15	4.7	8	1.5

8. *Correlation of mannitol clearance and urine flow.* The observation that the mannitol U/P ratio was relatively fixed over a wide range of filtration rates indicated a close correlation between mannitol clearance and urine flow, as illustrated in Figure 7. This suggests (*a*) that the rate of urine flow in shock depends chiefly upon, and is quite closely regulated by the rate of filtration, and/or (*b*) that the plasma concentrations of mannitol happened to be high enough and sufficiently uniform to impose upon the filtrate an increase in osmotic pressure, large enough to limit reabsorption of water to the point where the final urinary concentration of mannitol was always about 4 or 5 grams per cent. Since the primacy of the neurohypophysis in the regulation of facultative tubular water reabsorption seems so well established, the first mentioned possibility is believed untenable, except in a gross way. Hence, the correlation in Figure 7 may be considered what one might call an "osmotic accident." Furthermore, no close linear relationship between filtration rate and urine flow has been observed in the absence of mannitol, although, in general, greatly reduced rates of urine flow were associated with low filtration rates.

9. *Influence of etiological factors upon renal clearances.* No consistent differences have been found in the renal plasma clearances in patients

with skeletal trauma compared to those with hemorrhage. The tendency toward high filtration fractions in the head injury group has already been mentioned. That shock due to burns results in a distinctive renal clearance pattern is suggested by the data on the 2 patients shown in Table I. The filtration rate was disproportionately high in R. G. and probably also in J. R., yielding a high filtration fraction. A third case of burns recently studied showed an even more unusual filtration fraction of 41 per cent, with clearances of inulin and p-aminohippurate of 66 cc. and 159 cc. per minute per 1.73 square meters of body surface, respectively, measured when the blood volume was reduced to 1700 cc. per square meter of body surface, and the cardiac output to 1.83 liters per minute per square meter. The arterial mean pressure was maintained at the almost normal value of 75 mm. mercury (high peripheral resistance). These data were obtained prior to plasma therapy, suggesting that the fundamental pattern in the other 2 cases had probably not been greatly modified by the plasma administered.

10. *Influence of alcoholism on clearances in shock.* Because acute alcoholism of moderate or severe degree was present as a complication of trauma or hemorrhage in a substantial number of the patients, an attempt has been made to evaluate its influence. In alcoholics whose loss of blood volume was slight or moderate, blood pressure has been found to be significantly lower than in non-alcoholics having comparable blood loss and cardiac output reduction; *i.e.*, peripheral resistance was usually decreased (12). That there was a tendency for the kidney to participate in this relative vasodilatation is indicated by the observation that (*a*) the filtration fraction tended to be low (Table II), and (*b*) some alcoholics had renal blood flows considerably greater than most non-alcoholics with similar blood pressures. The data are inconclusive, however, because of an inadequate number of cases and the multiplicity of factors involved; hence only a general impression can be recorded.

11. *Influence of blood or plasma transfusion.* Transfusion of relatively large quantities of whole blood or plasma could affect renal filtration and blood flow (*a*) indirectly, by increasing the blood pressure, (*b*) by direct pharmacological action of substances contained in the blood which might alter the caliber of the renal vessels, or (*c*) by means of both. As with alcoholism, it is not possible to draw definite conclusions regarding the effect of transfusion upon renal hemodynamics. However, the following tendencies have been noted: (*a*) increase in filtration fraction, suggesting a relative constrictor action in the efferent arterioles, similar to most pressor drugs (see Table II); (*b*) increase in filtration rate concommitant with rising blood pressure; and (*c*) progressive fall in plasma flow, after an initial rise.[8] (See Figures 10, 11, and 12.)

12. *Clearances during immediate recovery from shock.* Perhaps intimately related to the influence of blood transfusion upon the renal circulation is the observation that in practically all cases adequately studied, the renal circulation failed to return to normal by the time the cardiac output and blood pressure had been completely or nearly restored. (See T. D., J. V., S. R., M. M., P. P., and A. S. in Table I and Figures 10 to 12.)

Up to the present, we have not studied the renal circulation during the days following injury and emergency treatment. However, of the several delayed deaths in this group, none could be definitely ascribed to renal failure. Practically all of the patients re-studied long after recovery from shock (see Table I and Figures 9, 10, and 11) showed normal filtration rate and renal blood flow.

[8] Quite similar results have been reported by Corcoran and Page after blood transfusion in dogs previously subjected to hemorrhage (14). Several explanations of our data may be suggested: (*a*) The bank blood may have contained variable amounts of vasoconstrictor substances (15); (*b*) it is possible that in shock there is an overproduction of renin which results in depletion of renin-activator (hypertensinogen), in which case the giving of blood or plasma would make available more activator, and the resulting angiotonin (hypertensin) could then exert a pressor effect generally and in the kidneys (16 to 18); or (*c*) there might be some autonomous intrarenal mechanism which reacts to rapid increases in blood pressure by constricting the efferent arterioles. In a preliminary study of the effect of blood transfusion (250 to 500 cc.) upon the renal circulation in normal subjects, Chasis *et al.* (19) found no change in the clearances or filtration fraction. This tends to weaken the first possibility cited above, although the amounts given may have been too small. Obviously, no definite conclusion is possible at the present time.

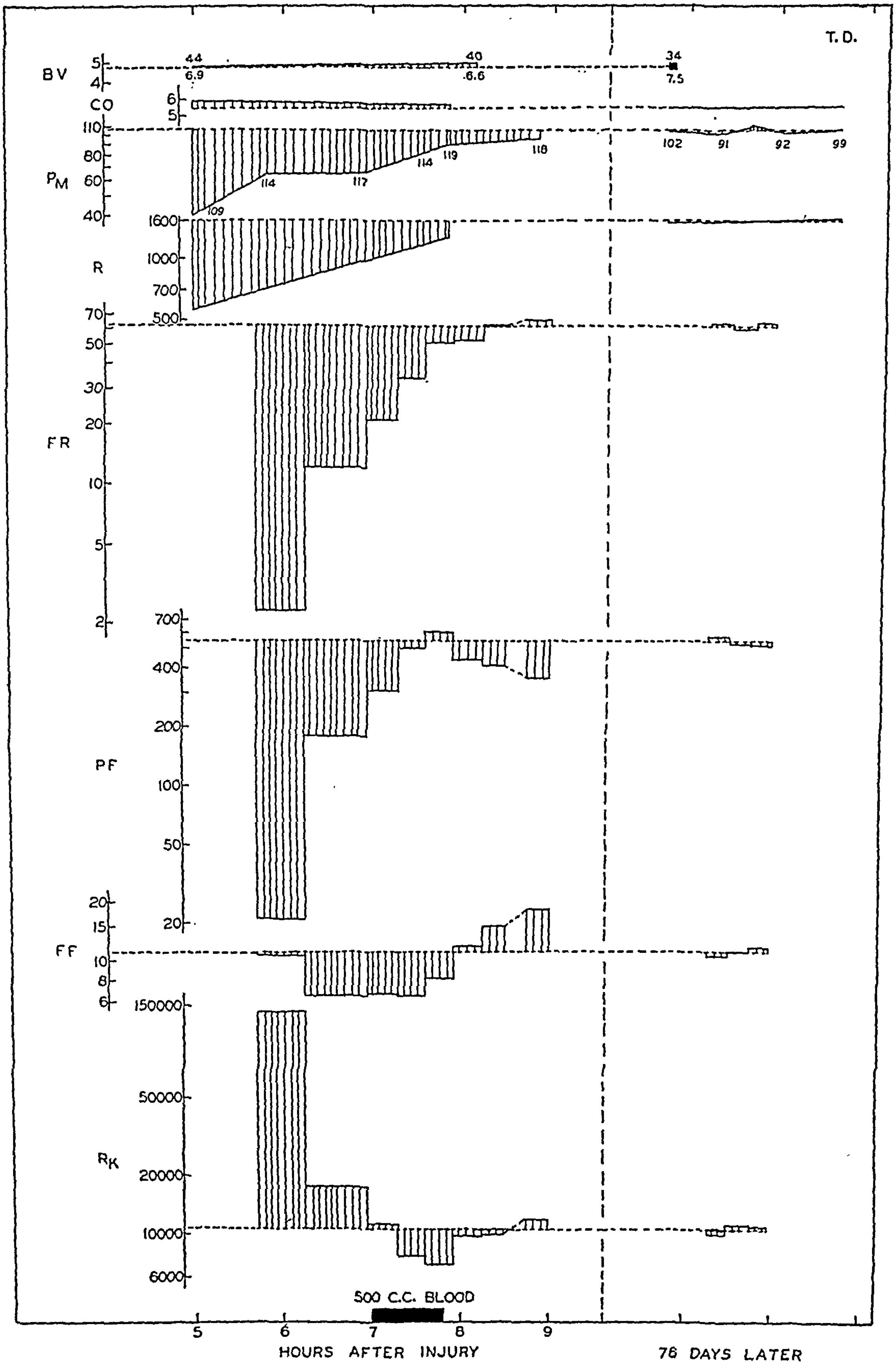

FIG. 10. T. D., 48-YEAR-OLD MALE. FRACTURES OF EXTREMITIES AND ACUTE ALCOHOLISM

Units of measurement as in Figure 8. Base-lines indicate values obtained in a repeated study 76 days later, at which time the patient was still in casts and in a relatively poor state of nutrition.

Note the striking initial reduction in blood pressure despite normal cardiac output (greatly reduced peripheral resist-

Details in 5 selected cases are illustrated in Figures 8 to 12. Many interesting aspects of the study may be seen in these charts. All explanations are given in the accompanying legends.

DISCUSSION

Limitations of the clearance methods in shock. In applying a method developed on normal subjects to an unexplored field, such as shock, it is essential to reiterate its limitations and to prove, if possible, its validity. For the historical development of the clearance technics and their general interpretation, reference may be made to the monograph of Smith (20). A detailed discussion of interpretations, particularly as applied to the problem of hypertension but applicable in many respects to the present problem of shock, has been presented by this author (21). Only the most important points can be considered here.

Some of the technical sources of error have been discussed previously, but the fundamental question remains: Does the shock syndrome, with its attendant oligemia, hypotension, anoxia, and acidosis, acutely alter the function of the glomerular membrane and/or the tubular epithelium in such manner that the clearances of mannitol or inulin and of diodrast or p-aminohippurate can no longer be considered identical to the rate of glomerular filtration and plasma flow, respectively? Since direct proof in man is lacking, only some of the possibilities and their consequences as applied to our data can be pointed out.

Decreased permeability of the glomerular membrane as a result of shock is quite unlikely. An increase in permeability would not invalidate the use of inulin or mannitol clearance to measure filtration rate. That the shock syndrome could alter the tubular epithelium to permit some back-diffusion is possible, but it seems unlikely that this could be large enough to alter seriously the general conclusions.

It is quite possible, however, that anoxia could impair the mechanisms by which diodrast and p-aminohippurate are excreted by the tubules, thus reducing the extraction from peritubular blood. A significant decrease in extraction ratio, and consequent error in measurement of total renal plasma flow, would be expected only in the lowest range of clearance figures. For example, hippurate clearances over 100 cc. per minute might, on the basis of such reasoning, bear about the same relationship to the true renal plasma flow as they do in normal subjects. On the other hand, a clearance of 10 cc. per minute might well represent only one-half or one-third of the true plasma flow, corresponding to decrease in extraction ratio to as low as 0.50 or 0.33.

Important support for this concept has been afforded by the experiments of Phillips and co-workers (22), who have kindly furnished the following summarizing statement: "Studies of the extraction of para-aminohippuric acid by the explanted kidneys of dogs subjected to hemorrhagic shock and traumatic shock have shown that a constant fraction of about 83 per cent of the hippurate is extracted from the plasma by the kidneys while the renal blood flow is above 10 per cent of normal. At flows lower than this the extraction of hippurate decreases. Thus in a typical case the extraction of hippurate was found to be 37 per cent when the renal blood flow was one per cent of the pre-shock flow. In this instance the renal blood flow calculated from the hippurate clearance, assuming an extraction of 83 per cent, was 0.48 cc. per minute, while it was 1.05 cc. per minute when calculated from the hippurate extraction. In general, the use of the factor 0.83 for calculating renal plasma flow from the clearance in dogs leads to only a small absolute error, although this may amount to a large percentage error when the flow is below about 5 per cent of the control value."

On the other hand, the recent report of Corcoran and Page (14) showed a variable decrease in extraction of diodrast in dogs following hemorrhage and transfusion, sometimes when the plasma flow, calculated on the basis of inulin extraction and excretion, was not below normal. Furthermore, these authors found evidence of storage of diodrast during hypotensive periods, the stored material being rapidly washed out during the first few minutes after rapid blood transfusion. The true filtration fraction (extraction ratio for inulin) decreased during bleeding and increased sharply during transfusion, findings which are not unlike those reported in the present communication.

In view of these limitations, caution must be exercised in the interpretation of the results. This may best be appreciated by reference to Figures 1 through 6, and visualizing the true values for total renal plasma or blood flow in the data having the smallest apparent values as being possibly 2 or 3 times as large as shown. It is important to emphasize, however, that the decrease in kidney circulation resulting from shock remains very large, all possible errors notwithstanding.

Regional vasoconstriction normally follows reduction in blood volume, and the data demon-

ance) and normal blood volume, a circumstance which makes the proper assessment of the degree of shock, if any, quite difficult. Blood pressure, clearances, and urine flow rose spontaneously, and the time lag between them suggests the possibility of prolonged "dead space time" in this case. The effect of the small transfusion was quite dramatic. There was progressive rise in blood pressure and renal clearances during and after the transfusion. The steady increase in filtration fraction was due to a relatively greater increase in filtration rate than plasma flow, the latter actually decreasing after an initial rise. When the patient was re-studied 76 days later, all measurements were normal except for an unexplained low filtration rate, and mild nutritional anemia.

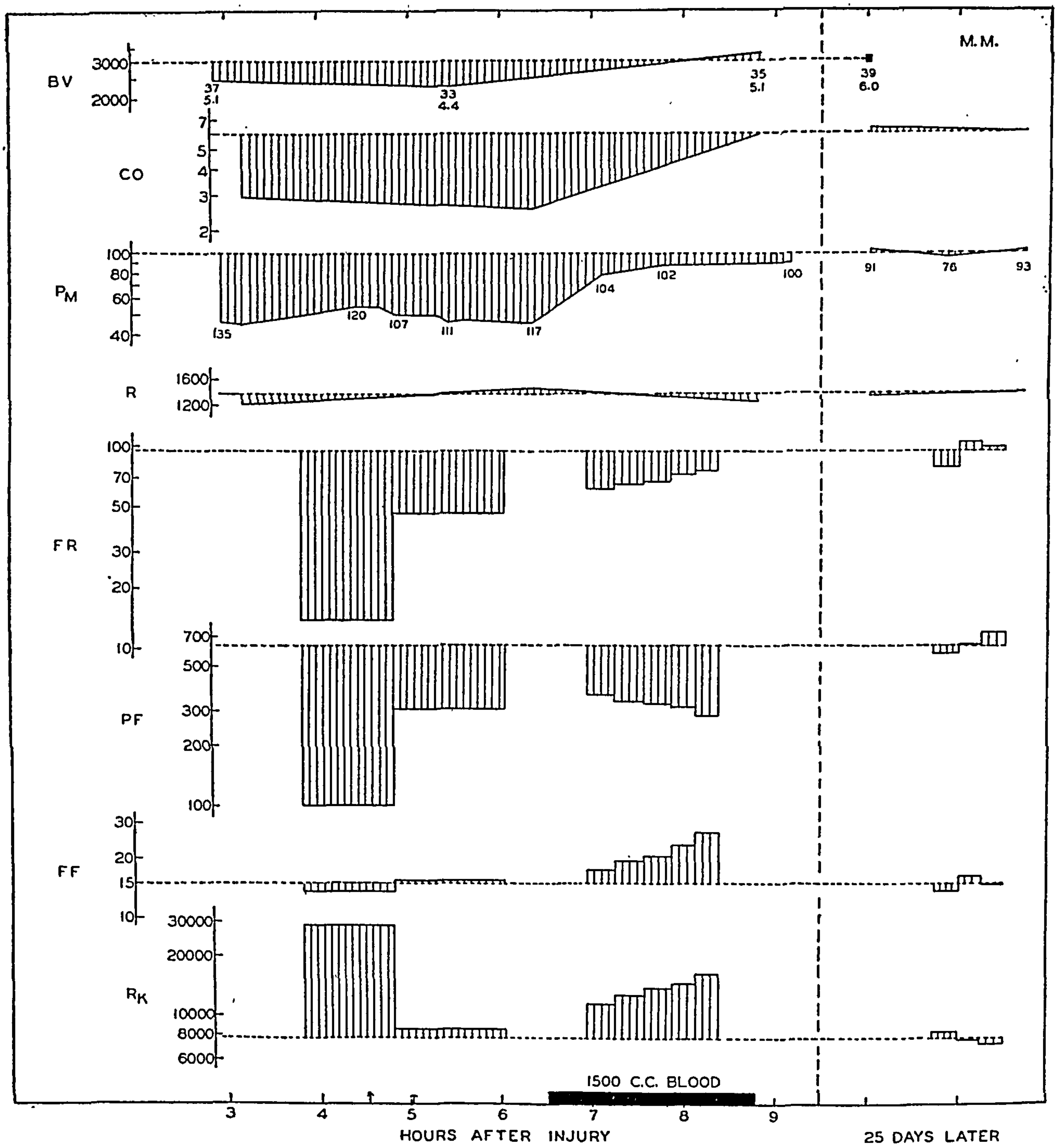

FIG. 11. M. M., 48-YEAR-OLD FEMALE. FRACTURED PELVIS WITH RETROPERITONEAL HEMORRHAGE. MODERATELY SEVERE SHOCK

See Figure 8 for units of measurement. Base-lines represent values obtained 25 days after injury.

Blood pressure and cardiac output were proportionately reduced and remained steady during the 3 hours preceding transfusion of 1500 cc. of whole blood. One hundred cc. of adrenal cortex extract intravenously (indicated by arrows on time scale) were without effect on the general circulation. Proportional increases in clearances and urine flow followed the administration of the hormone, not unlike those seen in J. S. (treated with cortical extract) and T. D. (no extract given), suggesting it was a spontaneous occurrence. The parallel increase in cardiac output, blood pressure, filtration rate, and filtration fraction during the transfusion, while the effective plasma flow decreased steadily, illustrates a response similar to that in T. D. This patient had consumed no alcohol. Note that in spite of the return to normal of the cardiac output and mean arterial pressure, the renal blood flow remained distinctly subnormal. Re-study 25 days later yielded normal measurements throughout, except for the blood volume which was considerably below "ideal" normal, and which probably is not a good index of the pre-shock value.

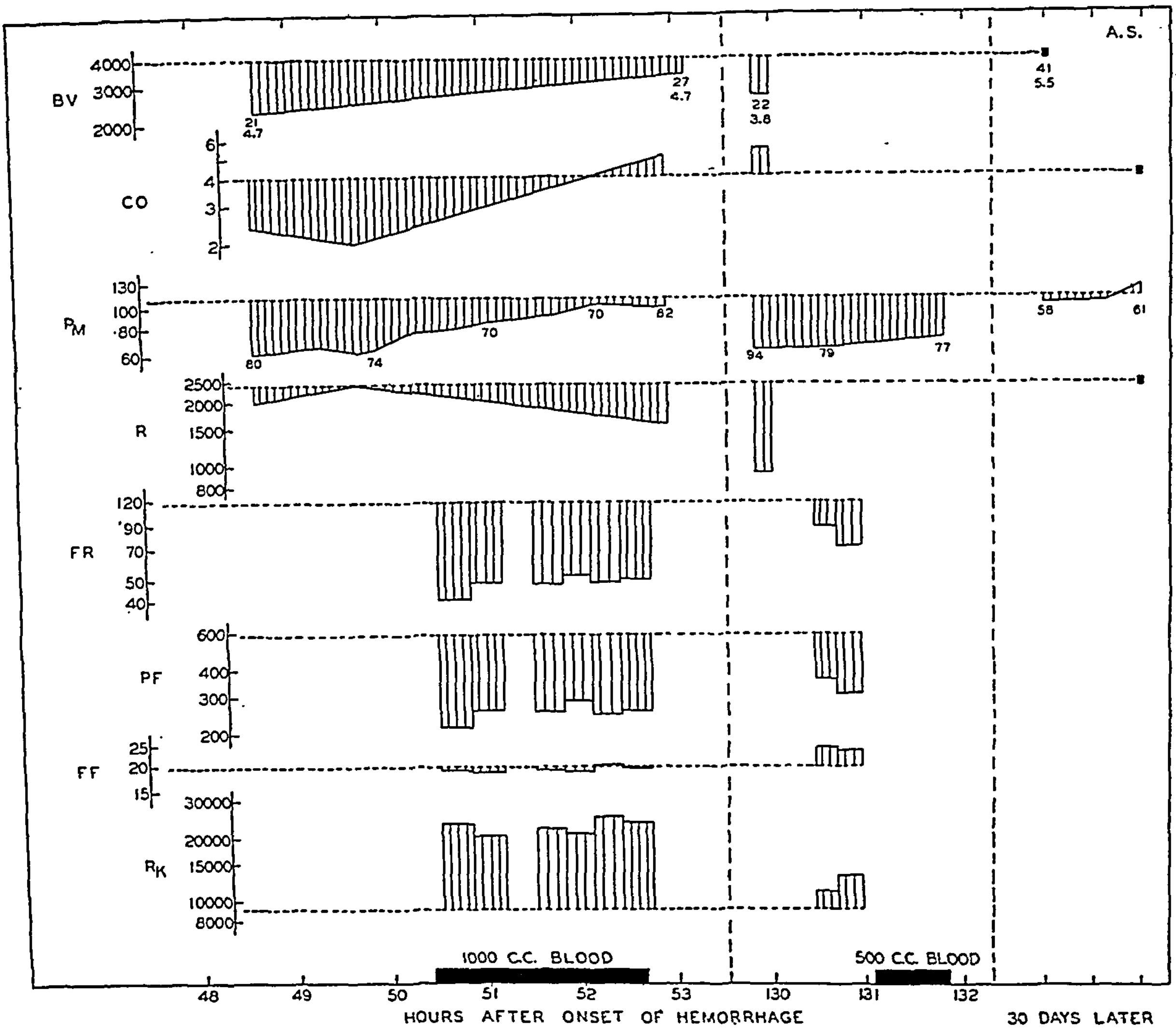

FIG. 12. A. S., FEMALE, AGED 60, VOMITED LARGE AMOUNTS OF BLOOD OVER A TWO-DAY PERIOD BECAUSE OF A BLEEDING PEPTIC ULCER

She was studied on the day of admission and again three days later. Units of measurement as in Figure 8. Baselines represent control values measured a month later, except in the case of renal clearances, for which average normal values were taken because no renal study could be obtained after recovery.

Cardiac output was reduced relatively more than the blood pressure, indicating high peripheral resistance. She was in moderately severe shock as judged by general appearance and low cardiac output. Response to 1000 cc. of blood was much less striking than in previous two cases as far as the renal values and blood pressure were concerned. The color improved remarkably, however, and the cardiac output rose to normal control levels. Filtration fraction remained unchanged, an observation which stands in contrast to the increasing filtration fraction following transfusion in T. D. and M. M. Repeated measurements 3 days later revealed interesting changes in the direction of decreased peripheral resistance, as well as decreased renal resistance, with maintenance of normal cardiac output. The renal clearances were distinctly improved at this time. The viscosity and osmotic pressure of the blood were very low (plasma protein, 3.8 grams per 100 cc.; hematocrit, 22 per cent), probably as a result of large quantities of parenteral fluids given during the preceding 3 days. There can be little doubt that these factors were of considerable importance in the reduction of peripheral and renal resistance, and may have been responsible for the increased filtration fraction at this time. After recovery from gastric resection performed after the second study, the patient was found to be mildly hypertensive. In view of the fairly good clearances observed on the third day, at which time the mean arterial pressure was at the relatively low level of 61 mm. of mercury, it seems probable that the hypertension had not caused significant permanent kidney damage.

strate that the kidney vessels participate in this constriction. The importance of renal vasoconstriction may be illustrated by Case G. J. (Table I, Figure 8): As a result of trauma, the total blood volume was reduced 30 per cent below the average normal value. The cardiac output decreased from 5.30 liters per minute (our average normal value for a man of 1.56 square meters body surface) to 3.75 liters per minute, a reduction of 1.55 liters per minute, or 29 per cent. At the same time, the renal blood flow was reduced from the average normal of 1000 cc. per minute to 160 cc. per minute. The net result was that the equivalent of 0.84 liter per minute of blood flow was spared for regions of the body more sensitive to anoxia, such as the brain. By the almost complete exclusion of the kidneys from the circulation by means of vasoconstriction, the blood flow to the rest of the body in this case was actually reduced by only 16 per cent, instead of 29 per cent.

The actual mechanisms involved in the reduction of renal circulation are, of course, not apparent from this investigation. It can be said that the renal resistance is increased in most cases, and that this is due chiefly to increased arteriolar constriction, probably afferent to a large extent, seems to be a reasonable conclusion. Whether the vasoconstriction in the kidneys is on a humoral or neurogenic basis, or both, cannot be answered, but it is clear that acidosis is not the initiating factor. The combination of acidosis and methemoglobinemia, recently shown by Bing (23) to be an effective means of producing a marked renal ischemia, cannot be invoked as an explanation of the renal vasoconstriction in shock, for reasons already stated, and because there was no hemolysis visible in the bloods of our patients.

The interesting observation that the renal circulation was only partially restored by amounts of blood which were adequate to increase the cardiac output to normal could be explained in several ways: (*a*) If the extraction of p-aminohippurate had been reduced during shock, it is possible that the tubule cells did not recover as promptly as the general circulation. This hypothesis would not account for the persistence of a subnormal filtration rate seen in several patients after transfusion (see especially J. V., S. R., and P. P. in Table I, and M. M. and A. S. in Figures 11 and 12). (*b*) The transfused blood might have contained pressor substances (15). (*c*) It is possible that the same mechanisms which produced regional vasoconstriction during shock persisted to some extent, possibly because the amount of blood administered was considerably less than that which was lost originally. This is in accord with the observations that a relatively small increase in blood volume often suffices to break the "vicious circle" (12), and implies a two-fold effect of blood transfusion, a blood volume increase and a vaso-pressor effect, which act synergistically to restore the circulation, at least temporarily. In essence, replacement of less blood than was lost appears to change an uncompensated oligemia, or shock, into a compensated one.

Until very recently, there seemed to be no need for special concern regarding recovery of kidney function in the treatment of shock due to trauma (crush injury excluded) or hemorrhage. Because no death had been clearly attributable to renal insufficiency, it was believed that transfusion therapy directed toward restoration of the general circulation was sufficient. However, in a recently studied case of traumatic rupture of the jejunum, renal function did not recover after transfusion, and renal failure with extreme oliguria, azotemia, and acidosis persisted for a week at which time the patient died from peritonitis. Among the possible factors, the duration of circulatory collapse prior to therapy, a considerably longer interval than in most previous cases, seemed the most likely explanation. Because of this experience, it is felt that transfusion must not only be adequate in quantity, but also should be instituted without delay to prevent extensive irreversible damage to the kidneys.

There remains the question of whether the vasoconstriction in shock is uniform throughout the kidney, or whether there is an irregular distribution. In the former, the blood supply to all nephrons would be equally reduced, whereas in the latter, some nephrons, or possibly whole anatomical units of nephrons, would be rendered ischemic. Temporary or permanent tubular damage would be expected only in areas receiving less than a minimal oxygen and blood

supply over a sufficiently long period. At present, there is little information bearing on this point, but the clinico-pathological study of Penner and Bernheim (24) favors the latter interpretation, since they found the distribution of ischemic cortical necrosis to be irregular in the kidneys of patients dying after prolonged peripheral vascular failure.

SUMMARY AND CONCLUSIONS

The changes in renal vascular dynamics resulting from peripheral circulatory failure have been investigated by means of the clearance methods in 35 human cases. The study was part of the comprehensive investigation of the circulation in shock carried on at Bellevue Hospital.

The following conclusions may be drawn:

1. The rate of glomerular filtration and effective plasma flow are reduced in practically every case of shock. The reduction is variable but roughly parallels the degree of shock.

2. In most cases, the decrease is greater than can be accounted for solely on the basis of reduced arterial pressure, suggesting active vasoconstriction in the renal vessels. The relationship between the renal blood flow and general circulation has been expressed in terms of two calculated values: The renal fraction, which designates the approximate proportion of total blood flow (cardiac output) which circulates through the kidneys, and the effective renal vascular resistance, which indicates the relation between systemic blood pressure and the renal blood flow. The decrease in renal fraction usually observed reveals that a smaller proportion of the cardiac output flows through the kidneys, indicating that blood is shunted away from the kidneys during shock. The increase in renal resistance indicates that renal vasoconstriction is the mechanism responsible for this redistribution of the circulation.

3. In general, the lowest clearances were associated with lowest blood pH values, but several lines of evidence indicate that acidosis is not the primary cause of decreased renal circulation. On the contrary, renal ischemia probably augments the acidosis resulting from widespread tissue anoxia.

4. Sources of error and limitations inherent in the clearance methods as applied to the study of shock are discussed. It is concluded that the clearances give a reasonably accurate description of the status of the renal circulation in this condition.

5. Urine flow is uniformly reduced and, in extreme cases, total anuria occurs. In general, the degree of oliguria reflects the reduction in rate of glomerular filtration, which in turn is related to the reduction in renal blood flow.

6. From a limited experience, the impression has been gained that acute alcoholism complicating slight or moderate oligemia tends to result in a relative generalized vasodilatation, which is reflected in the kidney by a lower filtration fraction and a renal blood flow larger than in nonalcoholics with similar levels of blood pressure.

7. The influence of blood or plasma transfusion upon the clearances has been studied. There is a tendency for the filtration fraction to increase, suggesting efferent arteriolar constriction. The filtration rate increases with the rise in arterial pressure, but the renal blood flow tends to remain low or to fall to subnormal values after a temporary increase.

8. In spite of the approximate return to normal of blood pressure and cardiac output, the renal circulation has, in most of the cases which have been adequately studied, failed to improve proportionately during the period of emergency treatment. However, measurements repeated several weeks later revealed a normal filtration rate and effective renal blood flow in all the cases so studied.

9. This investigation confirms the hypothesis that the urinary findings in shock, namely, oliguria or anuria, and loss or impairment of concentrating power, are the result of decreased circulation through the kidneys.

10. Therapy of shock in relation to the kidney is briefly discussed.

The authors gratefully acknowledge the cooperation of the other members of the shock study group, Drs. E. S. Breed, R. L. Riley, and R. P. Noble and Nurses Gertrude Hanafin and Dean Smith. We are indebted to Drs. Homer W. Smith, Dickinson W. Richards, Jr., and M. I. Gregersen for their guidance and helpful criticism.

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THE FATE AND EFFECTS OF TRANSFUSED SERUM OR PLASMA IN NORMAL DOGS

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INTRODUCTION

Since the recognition of the primary importance of restoring circulating blood volume in the treatment of peripheral circulatory collapse or "surgical shock," the search has been made for easily available substitutes for whole blood. Among the substances tried are solutions of crystalloids, gum acacia, or ascitic fluid, blood serum, and plasma. In the past few years, attention has centered almost exclusively on the use of plasma and serum. This is because they most nearly approach whole blood in physiological characteristics, obviate the necessity of cross-matching, and can readily be preserved for future use either in the original form, partially concentrated, or in the dry lyophile state.

The use of serum or plasma for restoring circulating volume is, of course, based on the osmotic effect of the proteins supplied. Volume gain will therefore be determined by the extent to which the protein provided is held in the vascular compartment. That, under various circumstances, protein may be rapidly removed has been shown by a number of experimental observations made within the past several years. Thus, Sibley and Lundy (1) at the Mayo Clinic found that 24 hours after a 500 cc. transfusion, the increase in circulating volume of patients accounted only for the added red blood cells, and that the added plasma of the transfusion had apparently disappeared from the circulation. Marriott and Kekwick (2) found similar results in transfusing anemic patients. Boycott and Oakley (3) and Krumbhaar and Chanutin (4) made the same observation on dogs. Freeman and Wallace (5) found that protein given as lyophilized serum was retained for 3 hours, but had disappeared in 24. Since the work reported in the present paper was done, both Beattie (6, 7) and Scharpey-Schafer and Wallace (8) have indicated that transfused plasma protein may leave the vascular system. J. D. Robertson (9), of the Middlesex Hospital in London, did comparative studies on the intravenous use of crystalloids, gum acacia, and serum in cats, and found, contrary to expectation, that the serum disappeared from the blood stream about as rapidly as did solutions of saline or glucose. Price and Metcalf (10), in the Hunterian laboratory at Hopkins, produced acute anoxia in dogs by removing red cells from the circulation, and, in order to maintain the normal circulating volume, introduced plasma in amounts corresponding to the volume of red cells removed. However, at the end of 6 to 8 hours, the circulating blood volume corresponded only to the original volume minus the volume of red cells removed. The volume of plasma which had been introduced to make up the difference had disappeared from the circulation.

In view of these observations and in view of the increasing clinical use of various types of human protein solutions (normal, concentrated, or lyophilized serum and plasma), and because of the great immediate importance of the subject in connection with the war, it was thought that a quantitative study of the effects of protein transfusions would be both interesting and timely. The experiments reported in this paper were therefore set up to establish a base-line on intact animals of the effects of the transfused protein solutions on the plasma and red cell volumes, total serum protein, protein concentration, blood pressure, kidney function, etc., and also to determine the fate of the water and protein transfused. The establishment of this base-line would simplify further quantitative work on the effects of protein transfusions in trauma, hemorrhage, burns, and other disturbances causing peripheral vascular failure.

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[1] This work was planned with the help of Dr. S. C. Harvey and Dr. P. B. Price and was done when the author was Harvey Cushing Memorial Fellow in Surgery, 1939–40.

[2] Now 1st Lieutenant, M.C., A.U.S.

METHODS

The dye method of Gibson and Evelyn (11) was used to determine plasma volume. The plasma volumes and protein concentrations were determined before and at given intervals after the transfusion. By multiplying the two together, the total protein at these points was found. Knowing the plasma volume and the relative volume of blood cells (measured by hematocrit), the total blood volume and the volume of red cells at these points could also be calculated. Thus, the effects on the circulating blood and its constituents of the addition of a known volume of plasma or serum containing a known amount of protein could be determined.

The experiments were performed on fasted mongrel stock dogs weighing 3 to 12 kgm. They were anesthetized with nembutal 35 mgm. per kgm. and the anesthesia reinforced with 10 mgm. per kgm., as necessary. The bladder was catheterized, and the urine collected in 4 batches; the first during the control period and the remaining 3 in approximately equal periods for the duration of the experiment after the protein transfusion. The carotid or femoral artery and accompanying vein were then exposed aseptically (the animals were used once or twice again later) and the artery cannulated. Connected to the cannula through one limb of a three-way stopcock was a mercury manometer recording mean blood pressures and a system which prevented clotting by feeding isotonic sodium citrate slowly into the cannula at a pressure slightly above the mean (usually no more than 4 to 5 cc. were used during an experiment). Blood specimens were taken from the cannulated artery by the use of two 5 cc. Luer-Lok syringes, one dry and one containing 0.5 cc. of 2.5 per cent sodium citrate, attached to a second stopcock fitting the open end of the first. By drawing about 5 cc. of blood into the syringe containing the citrate, the terminal portion of the artery and the stopcock-cannula system were cleared of stagnant blood. The specimen for the determinations was then taken with the dry syringe and the citrated blood in the first syringe was returned to the circulation. This method allowed accurately measured specimens, representative of the circulating blood, to be obtained quickly at any given moment and without any hemolysis or dilution from the blood pressure system (12).

After cannulating the artery, 3.0 to 5.0 cc. of a 0.10 per cent solution of the dye T 1824 was given intravenously. The following hour served as a control period during which the normal values for plasma, red cell, and total blood volumes, dye disappearance curve, protein concentration, hematocrit, hemoglobin, mean blood pressure, and rate of urine secretion were determined. Five and sometimes 6 blood samples were taken during this period, at 10-minute intervals, to ensure a fairly accurate determination of the rate of dye disappearance and of the plasma volume. Following this, the particular protein solution being tested, normal serum, normal plasma, or lyophilized serum 4-fold concentrated, was given into a convenient vein in a period of 10 to 20 minutes. Specimens were then taken at 15 or 30-minute intervals until the termination of the experiment. At this point, the volume was again determined by a reinjection of another 3.0 cc. of dye.

The specimens withdrawn, 4.5 cc. each, were divided into two portions, 2.5 cc. under oil for serum, and 2 cc. into bottles containing dry ammonium and potassium oxalate mixture. The serum from the clotted specimen after centrifuging was used for the measurement of the dye concentration, serum protein concentration, non-protein nitrogen, and chlorides. Blood cell volumes and hemoglobins were determined on the oxalated specimen.

The color intensities of the solutions containing dyes were measured by means of the micro unit of the Evelyn photoelectric colorimeter (13, a). The sera were analyzed for proteins in duplicate by the falling drop method of Barbour and Hamilton (13, b), and the concentrations were calculated by the formula of Weech (14). The cell volumes were determined by the use of Wintrobe hematocrit tubes, and the hemoglobins, with the photoelectric colorimeter. The non-protein nitrogen and chloride of serum and the nitrogen and chloride of urine were measured by the routine clinical laboratory methods.

The serum and plasma used were obtained by bleeding large healthy stock dogs. For the plasma, 10 cc. of 2.5 per cent sodium citrate were used for each 100 cc. of blood withdrawn. The 4-fold concentrated serum was made by the addition of 25 cc. of sterile distilled water to the powder representing 100 cc. of normal serum.[2]

CALCULATIONS AND GRAPHIC REPRESENTATION

The primary calculation of volume was made by the usual method from the extrapolated dye concentration value (Lo) and the factor for the dye solution used. Serial determinations of plasma volume after the primary determination were usually made by multiplying the original volume (minus the amounts removed in sampling) by the ratio of the expected dye concentration to the actual dye concentration at the given times wanted. The actual dye concentrations of the specimens were, of course, determined with the colorimeter. The expected dye concentrations are usually read off, for the given intervals of time, on a graphic logarithmic projection of the original disappearance curve. However, this latter procedure was not accurate enough for the purposes of these experiments, since the very possible small error of 4 to 5 per cent in drawing the curve through the original 5 dye determinations would introduce an error of 24 to 30 per cent at the end of 5 hours in the expected dye concentration values and therefore an equal error in the volume calculations.

A method of calculation was therefore worked out whereby the over-all dye disappearance rate

[2] Kindly supplied to us by Dr. John Reichel of the Sharp and Dohme laboratories.

could be determined for the whole period of the experiment from the point of the original volume determination to the point of the final volume redetermination by the dye reinjection. The rate of dye disappearance in percentage per hour is given by the formula,

$$100\left(1 - \sqrt[t]{\frac{\text{mgm. dye remaining}}{\text{mgm. dye injected}}}\right)$$

where t is the time in hours elapsed. The value for the known amount of dye given is corrected for the amount of dye removed in the 15 to 20 specimens. The amount of dye remaining is calculated from the plasma volume determined at the end of the experiment by a reinjection of dye and the concentration of dye in the plasma as determined on the specimen taken a moment before the reinjection.

Knowing the rate of dye disappearance, R, and the actually determined dye concentration at any point, C, the expected dye concentration at the end of a given time interval, t, will then be $X(100 - R^t)$. The successive serial determinations are then carried out each from the one immediately preceding, as in the following example. Given a plasma volume V, to determine volumes V_1 and V_2, at time intervals t_1 and t_2, when the dye concentrations read on the colorimeter are C, C_1, and C_2 and the volumes of plasma removed by sampling are s and s_1. Then

$$V_1 = (V - s) \times \frac{C(100 - R^{t_1})}{C_1}$$

and

$$V_2 = (V_1 - s_1) \times \frac{C_1(100 - R^{t_2})}{C_2}\ .$$

These calculations, although somewhat complicated, were made relatively easy by the use of logarithms.

Having determined the plasma volumes and knowing the relative cell volumes, the total blood volumes and red cell volumes are calculated. The changes in size of the individual red cells were deduced from the changes in mean corpuscular hemoglobin concentration

$$\left(\frac{\text{Grams hemoglobin per 100 cc.} \times 100}{\text{Cell volume per cent}}\right).$$

The average of the 5 or 6 determinations of the control period was taken as normal and the ratio of that to the values determined during the experimental period, expressed as per cent, indicated the change in size.

For purposes of comparison, the results were all calculated on a percentage basis and are thus shown graphically in the figures reproduced. The values for plasma volume, total plasma protein, volume of red blood cells, and plasma protein concentration just prior to the transfusion are taken as 100 per cent. The values for blood pressure, chlorides, and N.P.N.'s are recorded in the usual terms. The percentage change from the control period in the rate of urinary excretion is given as a horizontal line, since the rate was determined by dividing the volume produced in a relatively long period of time ($\frac{1}{2}$ to 1 hour) by that time. The line therefore represents the average rate for the period. The salient data in each experiment are given in Table I.

RESULTS

A. The fate of the transfused protein

The most striking phenomenon observed in these experiments was that the transfused protein or its circulating equivalent disappeared from the blood stream (Figures 1, 2, and 3). As shown below, this could not be accounted for in the urine as whole protein or its metabolic products. Presumptive evidence that the protein was not immediately stored or broken down in the liver was obtained when serum was transfused directly into the portal system of a dog. Here, the protein appeared in the general circulation in approximately the amount transfused and then disappeared from the blood stream at about the same rate as in the other experiments (Experiment 3). The possibility that the donor protein might have some individual specificity, and was therefore being taken up in the recipient dog as a foreign substance, was also entertained. However, when dogs were transfused with their own serum, obtained from them a day or two before and refrigerated until used, the results were the same as in all the other experiments (Experiments 7 and 8).

Striking, too, is the fact that the added protein, or its equivalent, disappears from the blood stream at a constant rate. The rate varied from animal to animal and ranged between 0.5 and 3.8 grams per hour. Calculated in relation

TABLE I

Experiments with plasma and serum transfusions in dogs

Number	Sex	Weight	State of animal	Material transfused	Plasma volume before transfusion	Volume transfused	Concentration of circulating protein	Protein concentration of solution transfused	Protein in circulation	Protein transfused	Calculated volume; percentage of original	Determined volume;† percentage of original	Total blood volume; percentage of original	Volume of red cells;** Percentage of original	Red cell size; percentage of original	Rate of urine secretion;‡ percentage of original	Excess urine volume	Rate of protein disappearance	Rate of protein disappearance (percentage of total)	Rate of dye disappearance
		kgm.			*cc.*		*grams per cent*		*grams*		*per cent*						*cc.*	*grams per hour*	*per cent per hour*	
1	F.	3.1	Normal	Plasma	108	95	5.76	5.86	6.2	5.6	188	168	126	76	95	2,210	50	1.4	11.6	16.1
2	F.	5.8	Normal	Plasma	284	250	6.40	5.66	18.2	14.2	188	184	150	109	95	1,500	49	3.8	11.9	17.5
3	M.	5.5	Normal laparotomy	Plasma via portal vein	299	200	6.70	5.34	20.0	10.7	167	146	110	75	86	1,000	51	2.5	8.1	11.4
4	F.	6.4	Normal	Serum	352	100	6.74	6.99	23.7	7.0	128	123	110	95	95	246	9	2.8	9.1	11.0
5	F.	6.8	Normal	Serum	277	160	6.44	6.08	17.9	9.7	158	152	129	125	94	500	10	1.8	6.5	8.2
*6	F.	7.1	Normal	Serum	519	130	6.01	6.33	31.2	8.2	125	120	112	118	94	1,430	65	1.6	4.1	9.2
7		7.0	Normal 11 days later	Dog's own serum	564	100	5.32	5.66	30.0	5.7	118	117	111	113	92	490	69	1.3	3.7	11.2
8	M.	8.4	Normal	Dog's own serum	361	100	5.64	5.95	20.4	6.0	128	129	117	106	94	1,040	66	0.6	2.1	5.9
9	F.	5.9	Pregnant	Serum	318	150	5.31	6.12	16.9	9.2	147	151	147	149	93	79	17	0.6	2.3	6.6
10		5.4	Post-partum 1 week	Serum	332	250	6.94	5.34	23.1	13.4	175	160	162	191	90	5,700	170	1.6	4.4	10.1
11	F.	6.7	Depleted by plasmapheresis	Serum	340	140	3.83	6.18	13.0	8.7	141	150	151	203	76	690	35	1.0	4.6	9.2
12		7.4	High protein diet, 1 month	Serum	379	160	5.64	6.07	21.4	9.7	142	135	149	212	93	260	11	2.0	6.4	9.5
13	F.	6.5	Depleted by plasmapheresis	Lyophilized serum	426	25	3.96	21.5	16.9	5.4	106	114	74	94	89	1,080	115	1.1	4.7	9.0
14		6.8	High protein diet, 18 days	Lyophilized serum	320	24	5.24	20.9	16.9	5.0	108	128	117	118	91	570	43	0.4	1.9	5.2
15	M.	4.1	Normal (Biopsy)	Serum	150	196	4.53	5.19	6.8	10.2	231		91	78	91			1.2	6.8	12.3
16	M.	4.1	Normal (Biopsy)	Serum	155	150	5.17	5.48	8.0	8.2	196	178	145	136	92			1.3	7.7	13.2
17	F.	12.0	Normal (Biopsy)	Serum	347	300	5.78	5.66	20.1	17.0	187	179	74	75	92			0.8	2.2	6.6

* Bracket indicates same animal was used in both experiments.

** Corrected for change in size of red cells.

† Immediately after transfusion; curve extrapolated to mid-point of transfusion period.

‡ For period one-half to one hour after transfusion.

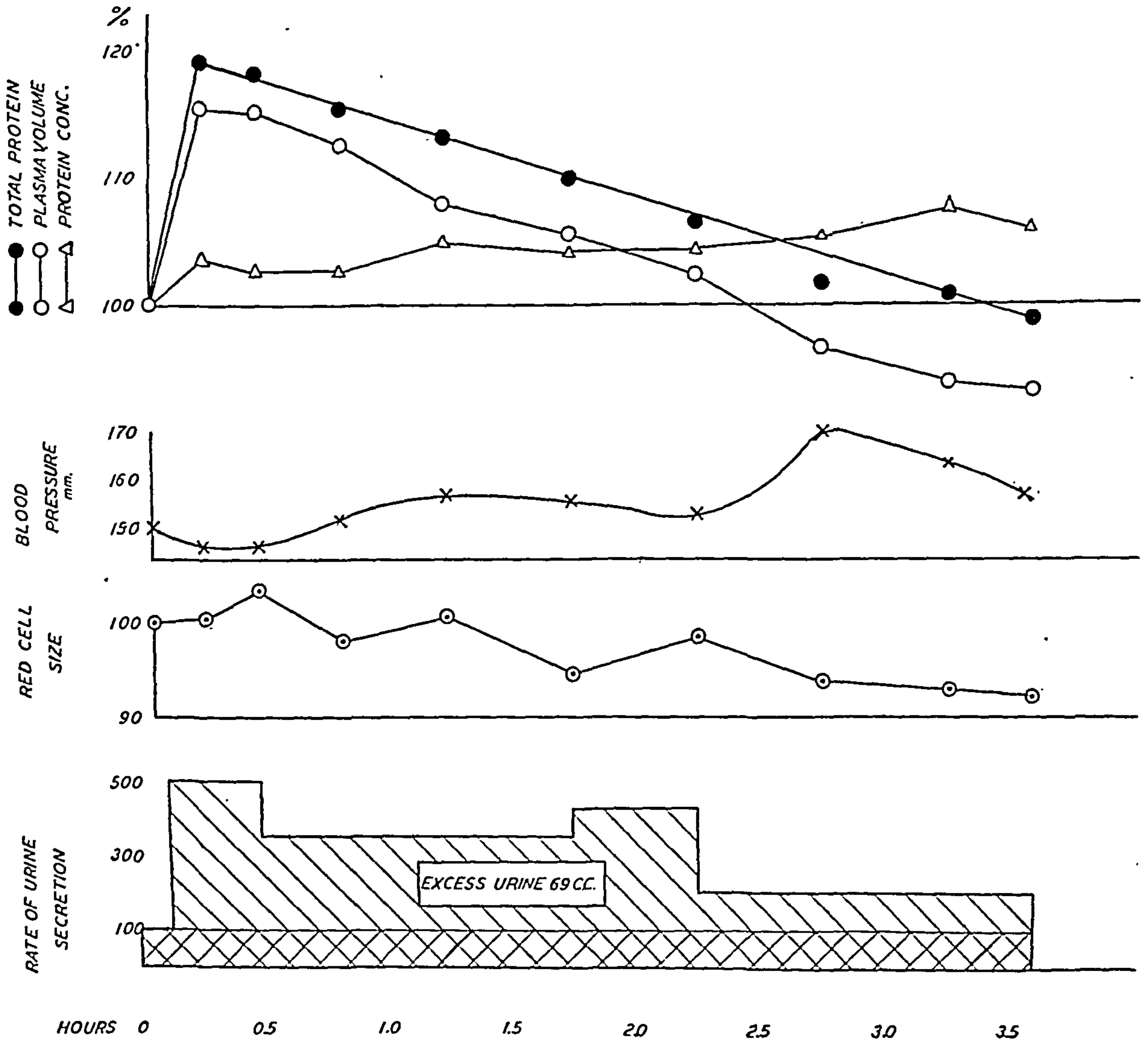

FIG. 1. ONE HUNDRED CC. OF NORMAL SERUM TO A NORMAL DOG (EXPERIMENT 7)
Note the reciprocal relationship between the protein concentration and plasma volume. Note too the increase in gradient of plasma volume change in the two intervals when the blood pressure rose.

to the total amount present, the rate ranged between 2.1 and 11.9 per cent per hour, with an average for the series of 6 per cent per hour. This rate is completely independent of the amount given as is shown by the fact that no correlation could be established between amounts given and rates of disappearance for the whole series of experiments (Table I). Neither was any correlation apparent between rate and volume transfused, surface area, depth of anesthesia, general condition, or age of the animal.

Very interesting, however, is the finding that there is an excellent degree of correlation ($r = 0.88 \pm 0.036$) between the rate of protein disappearance (in percentage of total per hour) and the rate of dye disappearance (Figure 6). Whether the rate of protein disappearance was logarithmic or linear could not be determined from the experiments, since for the magnitude of the percentage change per hour, the logarithmic and arithmetic projections both gave straight lines. Fortuitously, the experiments in which the rate may have been high enough to show a difference in the projection were done early in the work and only carried through for an hour or two (Experiments 1 and 2). Since the rate

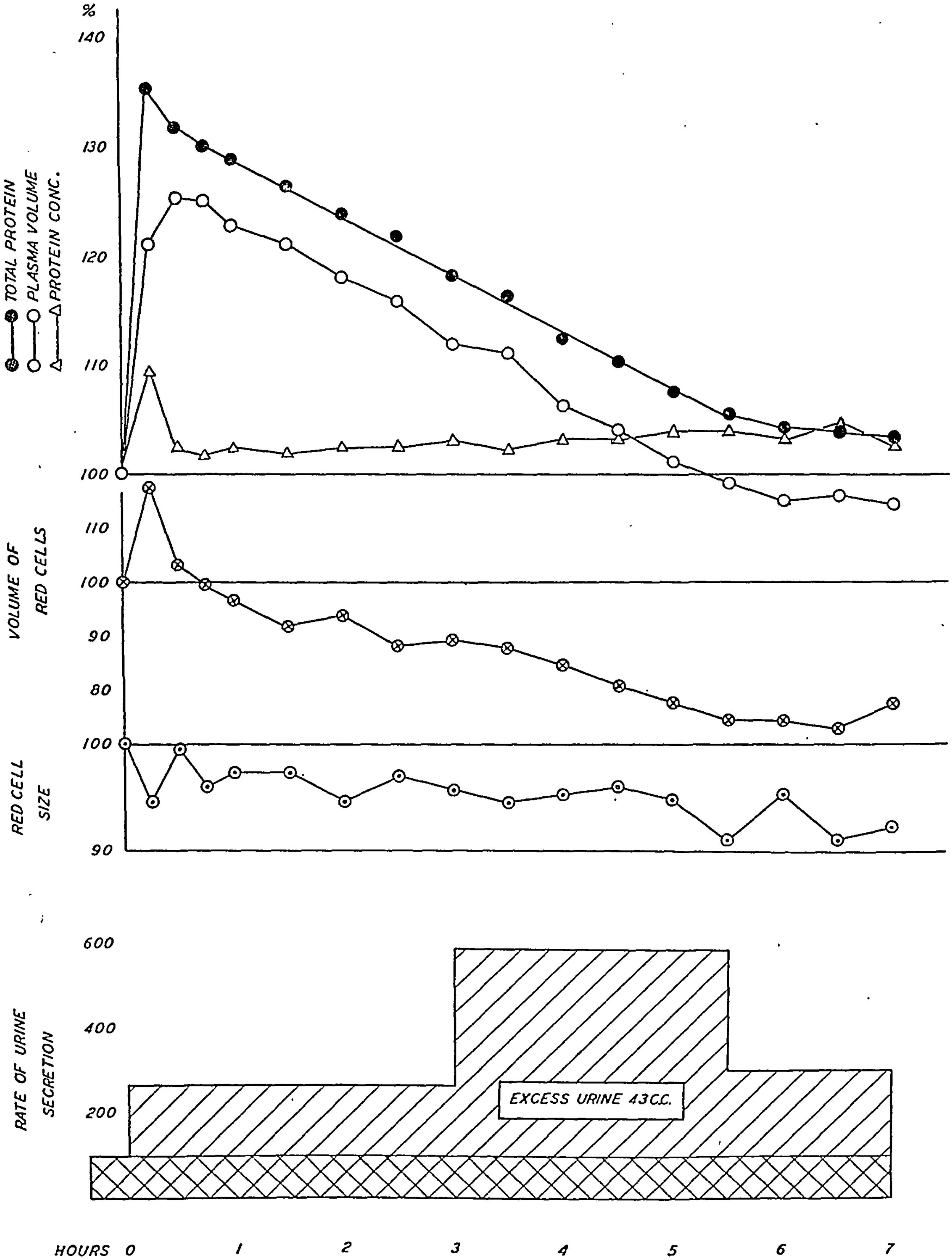

FIG. 2. TWENTY-FOUR CC. OF LYOPHILIZED SERUM (4-FOLD CONCENTRATED) TO A NORMAL DOG (EXPERIMENT 14)

The peak in the total protein curve represents 1.1 grams of protein coming into the circulation from extravascular sources and the hump in the plasma volume curve represents fluid coming into the circulation. (The blood pressure had dropped to about 60 mm. during the transfusion but came back to normal in the next 20 minutes.) The plasma protein and plasma volume return to normal in 5½ hours. Note that about 25 per cent of the volume of red cells (even with the correction for the change in size) has disappeared from the circulation.

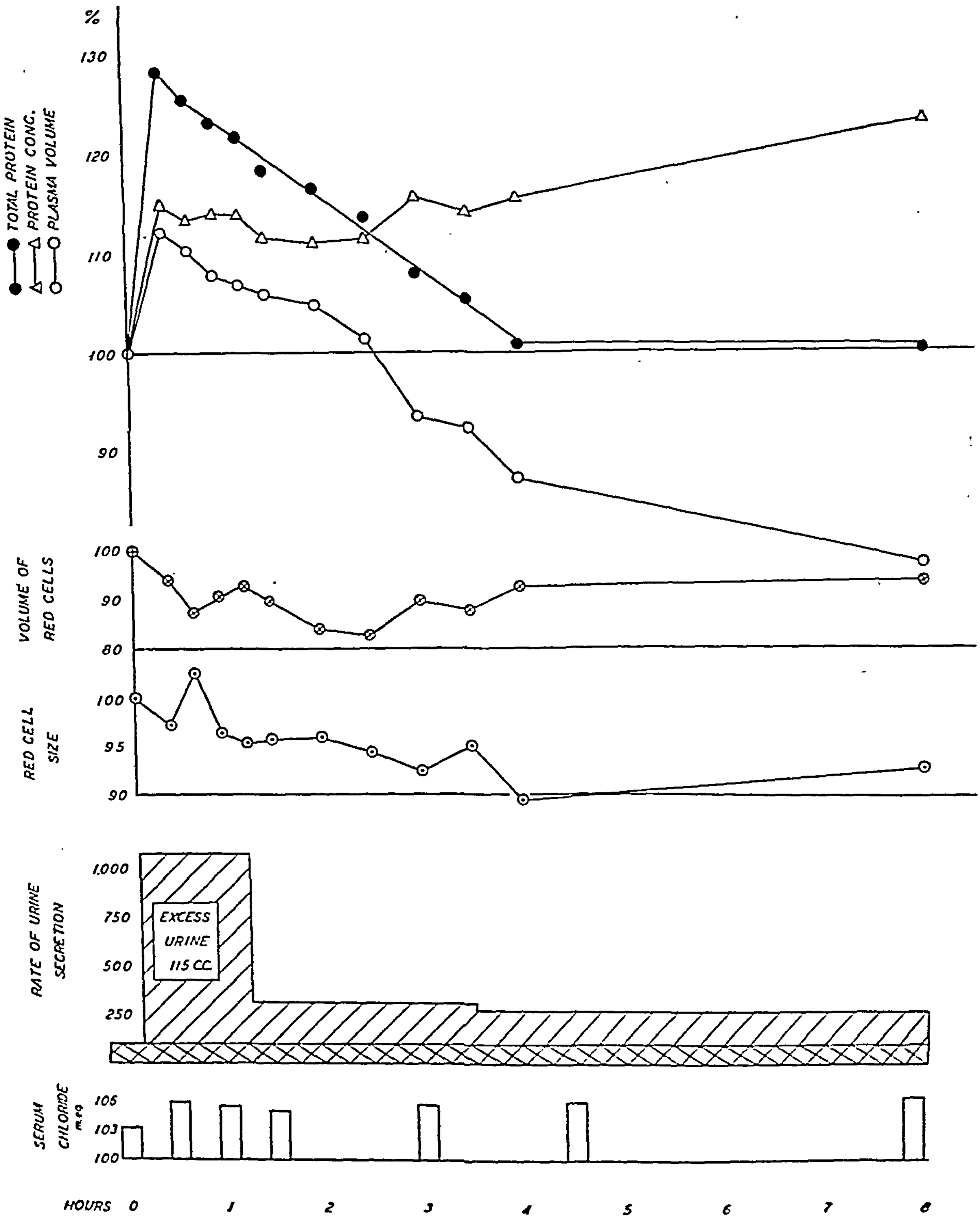

FIG. 3. TWENTY-FIVE CC. OF LYOPHILIZED SERUM (4-FOLD CONCENTRATED) TO A PROTEIN DEPLETED DOG (EXPERIMENT 13)

The total plasma protein returned to normal in about 4 hours and remained at that level while fluid continued to leave until the plasma volume was reduced to about 80 per cent of the original, with a resultant increase of the protein concentration. The urine volume secreted was almost 5 times the volume transfused.

of dye disappearance is logarithmic, the implication of the high degree of correlation is that the protein disappearance rate is logarithmic too.

To determine if the rate were dependent on the state of the "protein reserves," two animals were kept on a protein-free diet and depleted of protein to near edema levels by the plasmaphoresis method of Melnick and Cowgill (15). They were then transfused with both normal and lyophilized serum. The rates of disappearance in these two dogs were of the same order of magnitude as the average for the whole group and certainly not any greater. The protein levels and "reserves" were then brought back to normal in a few weeks by feeding a high protein diet after which the experiments were repeated. Here, too, the rate of disappearance was about the same and not any slower than when these animals were in a depleted state (Experiments 11, 12, 13, and 14).

The protein that leaves the blood stream is not excreted as such by the kidneys. Each of the 4 or 5 specimens of urine in each experiment was tested for albumin and in only 2 or 3 did a few specimens show just a trace. Neither is it excreted in the form of non-protein nitrogen. If all the excess non-protein nitrogen in the urine (the amount over and above that which would have been found had the rate of nitrogen excretion in the control period held throughout the experiment) is considered to have originated in the transfused protein, then for the group of experiments, only an average of 8 per cent of the amount given is so excreted. Of course, much or all of the latter may have been due to increased body protein metabolism because of increased muscular activity. The dog is quietest when in deepest anesthesia during the control period, just after the nembutal is given, and often during the experimental period, he shivers or struggles. There was also no increase whatever over the control levels in the serum N.P.N. in the 5 experiments in which it was determined at hourly intervals after the transfusion.

Immediate metabolism and excretion having been ruled out, the possibility of deposition of the added protein in a specific organ or tissue of the body suggested itself. In 3 experiments, liver, muscle, kidney, and intestine biopsies were taken before and 8 to 10 hours after a rather large serum transfusion. The specimens were then analyzed for water, chloride, and nitrogen. In these experiments, the amounts of water and protein remained remarkably constant but there was a slight drop in the chloride. If the protein had been taken up by any single organ, a determinable difference might have been found. However, the absence of a significant change suggests that the protein taken up was evenly distributed throughout the body. This would not be detectable by ordinary analytical methods, since the amount given averaged no more than 0.8 per cent of the total body protein. (The latter was calculated as 18 per cent of body weight on the basis of the analysis of animals done by Harrison, Darrow, and Yannett (16).)

Not only does protein leave the blood stream but a few of the experiments suggest the interesting possibility that protein may come rapidly, perhaps in limited amounts, into the blood stream from extravascular sources. This was seen in 3 experiments in which a sharp drop in blood pressure occurred during or immediately following the transfusion. In these 3, the total protein rose above the level determined after the transfusion and this rise amounted to 0.6, 0.7, and 1.1 grams, respectively. In none of the other experiments was a subsequent value for total protein greater than that determined immediately after the transfusion.

B. The fate of the transfused fluid

The transfused fluid, that is, the plasma considered without its protein, also leaves the blood stream and the plasma volume returns toward normal. This occurs in from 3 to 8 hours, depending on the rate at which the fluid leaves and on the amount given (Figures 1, 2, and 3). The rate of plasma volume change (fluid loss) is in almost all cases more rapid than the rate of protein loss. A concomitant upward trend in the plasma protein concentration results, but the latter did not go above 110 per cent of the original value except in one experiment in which lyophilized serum was used (Figure 3).

Although the rate of fluid loss is fairly constant, it varies from moment to moment within certain limits and causes an immediate reciprocal change in the protein concentration. The

latter goes up rapidly when fluid leaves the blood stream and goes down when fluid shifts in from the tissue spaces. These momentary changes in rate of fluid loss are synchronous with variations in the blood pressure. Throughout these changes, however, the rate of protein loss remains constant (Figure 1).

There was an initial shift of fluid into the blood stream only in two circumstances. First, when there was a sharp drop in blood pressure. This occurred in 3 or 4 experiments and was probably due to an anaphylactic type of reaction, as evidenced by the appearance of transient cutaneous wheals and the return of the blood pressure to the original level within an hour or so. Second, when the concentration of the protein in the material transfused was much greater than the circulating plasma protein concentration. This occurred in the two dogs which were given lyophilized serum and in the dog whose plasma protein level was 3.83 grams per cent and which was transfused with serum containing 6.17 grams per cent of protein (Experiments 11, 13, and 14).

Not all of the fluid transfused remains in the body with the protein; much of it is excreted by the kidneys. In all of the dogs, a greater volume of urine was excreted than would have been expected had the control rate of urine formation continued throughout the experiment. In 7 out of 14 experiments, the volume of excess urine was more than 50 per cent of the transfused volume. This was most striking in the two animals receiving lyophilized serum—approximately 5.5 grams of protein in 25 cc. of water. One dog secreted an excess of 115 cc. and the other, an excess of 43 cc. of urine (Figures 2 and 3).

The rate of urine formation ranged between 0.030 and 0.140 cc. per minute with an average for the series of 0.080 cc. per minute. This rate is tremendously increased during the first hour or so after the transfusion. It drops rapidly toward normal in the next hour or so and then approaches the control rate as the plasma volume returns to normal. The average increase for the group of experiments in the post-transfusion period was 680 per cent. The increase in rate in the post-transfusion period correlated fairly well with the percentage increase in the plasma volume, induced by the transfusion.

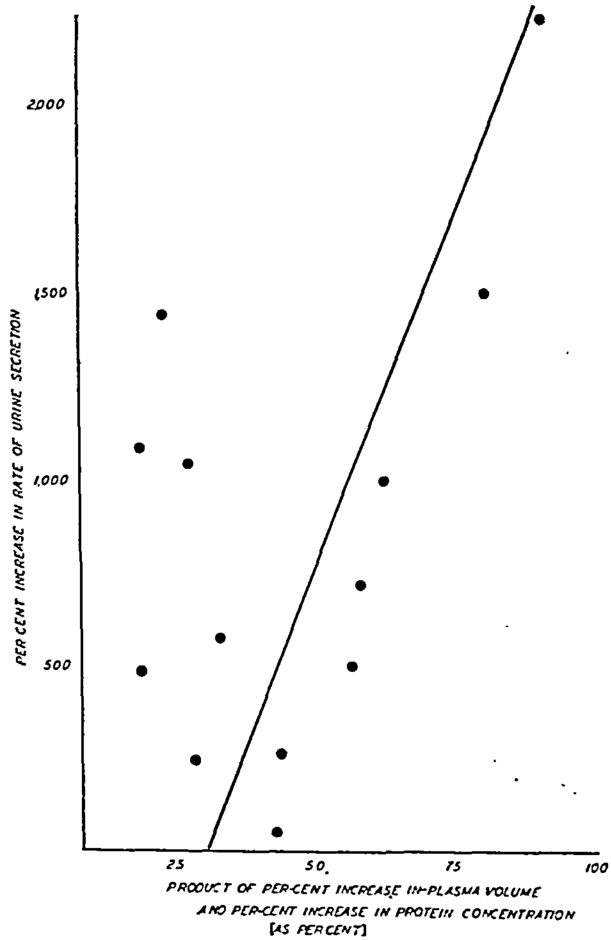

FIG. 4. THE CORRELATION BETWEEN THE PERCENTAGE INCREASE IN RATE OF URINE SECRETION FOR THE PERIOD IMMEDIATELY FOLLOWING THE TRANSFUSION AND THE PRODUCT OF THE PERCENTAGE CHANGES IN PLASMA VOLUME AND PROTEIN CONCENTRATION

This product expressed arbitrarily as per cent gave a better correlation than either the increase in volume or change in protein concentration individually.

The correlation was better when the change in protein concentration was also taken into account by multiplying the percentage volume increase by the percentage change in protein concentration (Figure 4). In those dogs where there was an initial drop in blood pressure, the diuresis occurred later in the experiment, when the pressure returned to normal. In only one dog was there a diminished rate of secretion throughout the experiment. This animal was pregnant and near term. Interestingly enough when the experiment was repeated on this same

dog a week or so post-partum, the rate of urinary secretion for the first hour after the transfusion was 57 times greater than that of the control period.

C. Other effects of the transfusion

The effects of the transfusions on circulating red cell volume were variable. In 8 of the experiments, there was a fairly marked increase, averaging about 50 per cent; in 5, there was little or no change; and in 4, there was a fairly marked drop, averaging about 25 per cent. In those where it increased, there was a gradual return to normal with the plasma volume; in those where the transfusion caused cells to leave the circulation, there was little or no tendency for a return to the original level (Figures 2 and 3). In general, the volume of red cells coming into the circulation was roughly proportional to the volume of added plasma, with the result that the percentage increase in total blood volume was approximately that of the percentage increase in plasma volume. The effect was therefore as though a volume of whole blood, approximately twice the volume of plasma, were

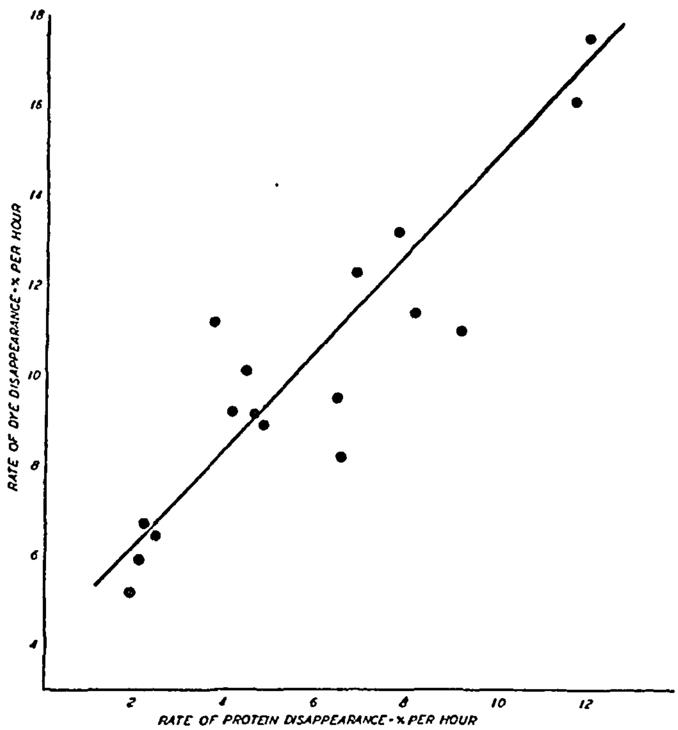

FIG. 6. THE CORRELATION BETWEEN THE RATE OF PROTEIN DISAPPEARANCE (AS PER CENT OF TOTAL CIRCULATING PROTEIN AFTER THE TRANSFUSION) AND THE RATE OF DYE DISAPPEARANCE

given. (Compare the eighth and ninth columns of figures in the table.) Of course, where red cells left the blood stream, the net effect on the total blood volume was the algebraic summation of the volume of plasma added and the volume of red cells leaving.

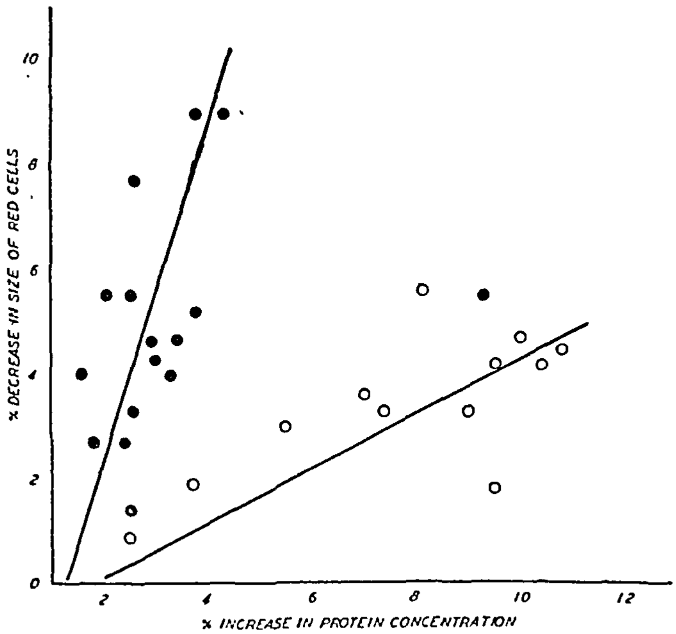

FIG. 5. THE CORRELATION BETWEEN THE DECREASE IN SIZE OF THE RED CELLS AND THE INCREASE IN CONCENTRATION OF THE PLASMA PROTEIN IN TWO OF THE EXPERIMENTS

The solid circles represent Experiment 7 (Figure 1) and the open circles Experiment 14 (Figure 2).

Although the total volume of red cells was somewhat variable, there was a constant change in the individual red cells. They decreased slowly but progressively in size throughout the post-transfusion period (Figures 1, 2, and 3). This is shown by the fact that there was a progressive increase in the mean corpuscular hemoglobin concentration. If the ratios of the control value to the post-transfusion values are taken as a measure of this decrease in size, the maximum change noted in each experiment ranged for the series between 5.0 and 23.6 per cent, with an average for the series of 8.3 per cent. In all probability, this decrease in size of the red cell indicates a loss of water from the cell. In about half of the experiments, there was a direct correlation between the percentage decrease in size and the percentage increase in plasma protein concentration (Figure 5).

Only two chemical constituents of the blood were studied—the serum non-protein nitrogen and chloride. Since the chloride concentration of the plasma or serum introduced was about the same as that of the circulating plasma there was of course no difference as a result of the transfusion. Where concentrated chloride was given in the lyophile experiments, there was a rise of only 2 to 3 m.eq. Excess chloride was excreted in those experiments in which there was a marked excess of urine put out and in approximately the same concentration as the control. There was nothing unusual noted in the distribution or excretion of the added non-protein nitrogen.

Finally, it should be noted that the changes in total blood volume, incident to the transfusion, caused no change in the blood pressure. The blood volume increases ranged between 10.0 and 84.0 per cent but in no single experiment was there as much as a 5 mm. increase in the mean arterial pressure. On the other hand, neither was there a significant drop in blood pressure when the blood volume decreased by 26 per cent, as it did in 2 experiments.

DISCUSSION

On the basis of nitrogen balance studies, Whipple (17) formulated the theory that "a steady state or ebb and flow exists between the plasma proteins and a portion of cell and tissue proteins." This steady state he referred to as "a dynamic equilibrium." More direct evidence for this theory was found by Schoenheimer (18) in his studies of metabolic processes with substances labelled by isotopic atoms. The results of the experiments reported in this paper are further confirmation of this theory, since they too show that protein not only leaves the blood stream, but may also come into it from extravascular sources.

If this state of equilibrium exists between most of the tissue proteins and the plasma protein, the explanation for quantitatively little or none of the protein transfused remaining in the blood stream becomes apparent. A 10-kgm. dog would have approximately 1800 grams of tissue protein and 30 grams of plasma protein. Therefore, any amount added to the latter might be expected to distribute itself in approximately the ratio of 60 parts in the tissues and 1 part in the plasma. Although actually this ratio may not be of the magnitude given, such a distribution undoubtedly occurs.

This helps to explain why patients with nephrosis or nutritional hypoproteinemia do not get an appreciable increase of plasma protein, even when given large quantities of protein intravenously. It also helps to explain why in many acute pathological conditions such as trauma, burns, or acute intestinal obstruction, plasma transfusions, even if large, may only be of temporary benefit. Fine and Gendel (19) found that if in shock due to experimental intestinal obstruction, they gave even as much again or more than the original volume of plasma, there was nevertheless a subsequent drop of plasma volume of 39 per cent and the dogs died in 5 or 6 hours. Rhoads, Wolff, and Lee (20), in a study of burn patients, noted that "when large plasma transfusions were administered soon after the receipt of a burn, there was not as great a rise in plasma volume as had been anticipated and furthermore, as a rule the rise that was obtained was temporary." That plasma was not only being lost locally by exudation from the burned surface, but that a good proportion of it was simultaneously leaving the vascular system, generally seems to be the most probable explanation for these observations.

The finding that the protein which disappears from the blood stream is not broken down and excreted by the kidneys had already been made by Howland and Hawkins (21) on phlorizin diabetic dogs. They concluded that the protein was removed from the blood stream and stored in the body tissues as such or that it was only partially broken down and then rebuilt into tissue protein.

The diuresis induced by the transfusions is probably not the result of increased osmotic pressure only, since it occurred in many experiments where no increase in protein concentration was caused by the transfusion. Weech (22) also found this to be true, and showed that a diuresis could be induced by giving a volume of red cells washed free of their plasma. He therefore concluded that the increased blood volume was the stimulus for the diuresis. In these ex-

periments, the increase in rate of secretion immediately following the transfusion was found to be roughly proportional to the increase in plasma volume and the change in protein concentration. The excess urine volume, in relation to the volume transfused, was greatest, however, in those cases where lyophilized serum was used. The possibility of causing dehydration or increasing that already existent with the use of concentrated or even normal plasma therefore should be kept in mind.

The decrease in size of the red cells also indicates a loss of water. The volume given up, however, is small in comparison to the excess volume excreted by the kidneys and the remainder of the latter must have come from extravascular sources. The correlation between the decreasing size of the red cell and the increasing protein concentration suggests that the former is due to increasing osmotic pressure. However, the change in size of the red cell is much too great to be explained on the basis of the observed change in protein concentration only, and therefore some of the electrolytic components of the plasma must have increased in concentration too. In those experiments where there was little correlation between the two, there may have been a decrease in electrolyte concentration, offsetting the effect of the increase in protein concentration.

It should be emphasized in passing that this change in size of the red cell may be one of the sources of error, and perhaps a major one, in the use of the hematocrit for the calculation of changes in total blood volume and volume of red cells, following plasma transfusions. This factor may explain many of the discrepancies noted in the literature between calculated and determined volumes of plasma and red cells on the basis of the hematocrit. Thus, the shrinkage of the red cells by 8.5 per cent (the average found in this series) will introduce a change of 15 per cent in the hematocrit and therefore an apparent discrepancy of 15 per cent between the expected and determined volume of red cells.

The exact mechanism by which the protein leaves the blood stream was not elucidated by these experiments. However, it was recently shown by Dawson (23) and Gregersen and Dawson (24) that the dye is bound to the albumin fraction of the protein. They concluded that "one implication . . . is that the rate of disappearance of T-1824 during the first hour after the injection is a measure of the rate of exchange of albumin" (between plasma and tissues).

Since the dye leaves at a logarithmic rate and there is a high degree of correlation in these experiments between that rate and the rate of protein disappearance, the conclusion is warranted that at least the albumin fraction of the protein transfused leaves at a logarithmic rate. The rate is therefore related to the total amount present, as is true of many other biological processes. Although the determination of albumin-globulin ratios was not done, it is probable that the added globulin also leaves the blood stream, since in those experiments which were carried far enough, all of the added protein disappeared, and the total protein remaining leveled off at the original amount present.

CONCLUSIONS

1. Protein given intravenously to normal dogs, either as normal or concentrated serum or plasma, leaves the blood stream in a relatively short time.

2. The rate of disappearance of this transfused protein is constant and not related to the changes in volume or concentration caused by the transfusion or to the state of protein reserves.

3. The correlation between the rate of protein disappearance and the dye disappearance suggests that the former is logarithmic and therefore related to the total amount of protein in circulation.

4. The diuresis induced is roughly proportional to the increase in plasma volume and change in protein concentration.

5. The transfusion causes a loss of water from the red cells resulting in a decrease in their size. The latter may be an important source of error in calculations of volume change by the hematocrit.

6. These experiments offer an explanation for the observation that, in many pathological states, only temporary benefits are derived from plasma transfusions.

7. These experiments add evidence, too, to the concept of a dynamic equilibrium between plasma and tissue proteins.

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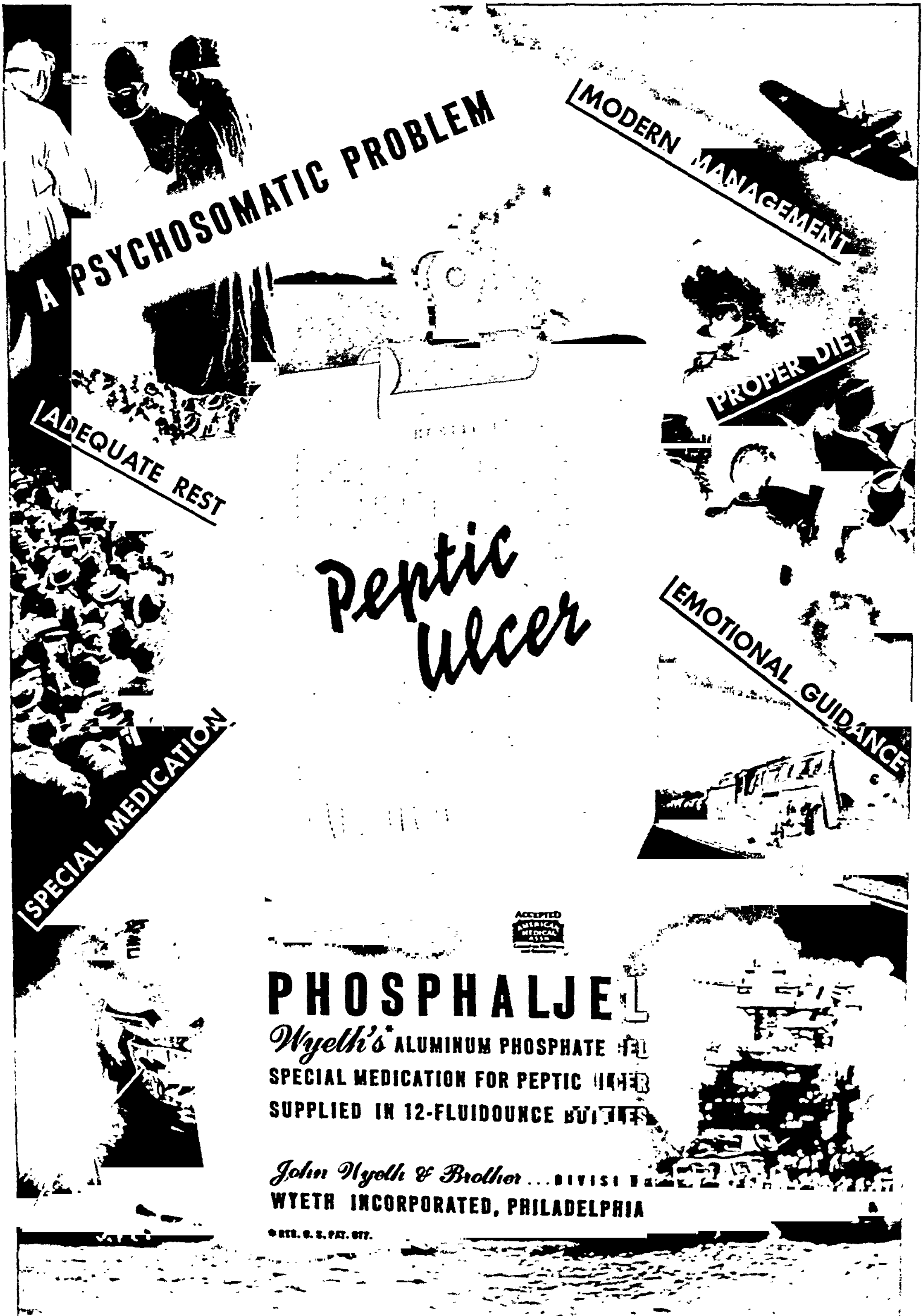
A PSYCHOSOMATIC PROBLEM
MODERN MANAGEMENT
PROPER DIET
ADEQUATE REST
EMOTIONAL GUIDANCE
SPECIAL MEDICATION
Peptic Ulcer
PHOSPHALJEL
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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

I. THE CHARACTERIZATION OF THE PROTEIN FRACTIONS OF HUMAN PLASMA[1,2]

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The object of the series of investigations herewith reported has been to determine how blood plasma, fractionated into its component parts, may be used with the maximum effect in the treatment of clinical conditions. Extensive experience has demonstrated that plasma is of unquestioned value in the treatment of shock, burns, and diseases in which there has been depletion of one or another of the components of plasma. Moreover, either pooled or convalescent plasma has been injected in man because of either proven or implied effectiveness in the prophylaxis or treatment of certain infectious diseases.

The constituents of plasma are not all equally effective in the treatment of diverse conditions, however. Therefore, the utilization of the whole plasma in therapy may often prove both less effective and less economical than the use of parts thereof. In the treatment of infectious disease, for example, such antibodies as may be present in blood constitute only a small fraction of the plasma globulins. The rest of the plasma has little proven value in the prevention or modification of contagious diseases. In the control of a measles epidemic, injection of the albumin of the plasma, or of the fibrinogen or prothrombin, would appear to serve little purpose.

Conversely, in shock, injection of the human antibodies, though they exert some colloid osmotic pressure, would be far less effective and economical than the injection of an equal amount of albumin. Twice the amount of the immune serum globulins—present in but small amount—would be necessary to produce the same colloid osmotic pressure as the albumin. Albumin is responsible for nearly 80 per cent of the colloid osmotic pressure of the plasma and blood and is thus responsible to a far greater extent than other constituents of the plasma for the maintenance of blood volume.

The significant functions of the blood are by no means all performed by the plasma. The respiratory function of the blood is carried out by the hemoglobin, the carbonic anhydrase, and other proteins within the red cells; and the hematopoietic function of certain of these proteins has been repeatedly claimed. Though such studies are in progress, the present series of communications makes no contribution to the important problem of the conservation of the valuable labile cellular constituents of the blood. As in the case of the plasma, however, it is possible that technics for the concentration and preservation of these physiologically active substances would lead to further therapeutic advances.

The number of components that may be identified in the plasma proteins, either by chemical, physiological, or immunological criteria, remains far greater than the number of fractions into which it has been convenient to

[1] This work was originally supported by grants from the Rockefeller Foundation and from funds of Harvard University. It was aided early in 1941 by grants from the Committee on Medicine of the National Research Council, which included a grant from the American College of Physicians. Since August, 1941, it has been carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[2] This paper is Number 13 in the series "Studies on Plasma Proteins" from the Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross.

[3] Welch Fellow in Internal Medicine of the National Research Council, Member, Society of Fellows, Harvard University, during the first years of these investigations.

separate the plasma proteins. Certain of the components are present in amounts so small that they cannot effectively be demonstrated in the whole blood. When they are separated from each other, concentrated, and made available in the dry state, or redissolved to yield stable solutions, their properties and functions can be investigated, and, in many cases, the products can be utilized. Among the constituents of plasma that have thus far been concentrated are the antibodies against infectious diseases, two of the components of complement, the anti-A and anti-B isohemagglutinins and Rh antibodies, hypertensinogen, thyrotropin and, of course, fibrinogen and thrombin, and the various groups of electrophoretically defined globulins and albumins.

Chemical characterizations are here reported of those products for which clinical appraisals either have been completed or are under way and which are now being produced in considerable amounts as part of the program for the plasma fractionation of Red Cross blood for the armed forces. These include (*1*) normal human serum albumin for use in shock and burns, (*2*) immune serum globulins for use in measles prevention and modification, (*3*) isohemagglutinins for use in blood grouping, (*4*) thrombin used with (*5*) fibrinogen for the formation of clots in certain surgical conditions including skin grafting and coagulum pyelolithotomy, (*6*) fibrin foams, prepared from fibrinogen and thrombin for use as hemostatics, (*7*) fibrin and fibrinogen tubes and plastics prepared to determine their most valuable surgical uses, and (*8*) fibrin films thus far used as a covering for burns and more recently as a dura substitute in neurosurgery.

The plasma fractionation program may from one point of view be compared with the early development of aniline dyes from coal tar. Until the intermediates were available, the cost of the preparation of any single product might have been considered prohibitive. After the intermediates were available, one could not afford not to develop the various products that could be derived from them.

The chemical methods that have been developed for large scale plasma fractionation, and which are now in nationwide use in the preparation of normal human serum albumin for the United States Navy, are not considered in this report.

In following the fractionation of a system as complex as blood plasma, it has become convenient to characterize protein molecules on the basis of behavior involving their size, shape, and electrical charge, rather than on the basis of specific reactive groups which may be involved in a given physiological function. The analytical methods used were chosen to be convenient, reproducible, and rapid. The value of the fractionation depends upon the correlation between the physiological properties and the components revealed by the analytical procedures. The first aim of the process was thus to separate and concentrate proteins of defined chemical properties into various fractions. These initial fractions were then tested for physiological activities. In actual practice, several analytical methods have been employed, and certain physiological reactions which could be followed simply have also been of considerable aid in developing the fractionation scheme.

This, and the papers which follow, are wholly concerned with the characterization of the products and the appraisal of their uses. The availability of these human proteins in large amounts opens diverse possibilities for the use of concentrated physiologically active components of plasma, both in surgery and in medicine.

THE PHYSICAL CHEMICAL METHODS EMPLOYED IN THE CHARACTERIZATION OF THE PLASMA PROTEINS

The aim of our plasma fractionation program has been to separate the components responsible for each function, and the aim of our physical chemical studies has been to evaluate the properties of the molecules as a means to this end. Certain properties are, moreover, of direct physiological importance. Thus, diffusion through membranes, resistance to flow through capillary vessels, and distribution of water among various body tissues are directly related to the size, shape, and electrical charge of the molecules involved. Indeed, many of the pioneer experiments in the fields of osmotic pressure, viscosity, and diffusion have been carried out by physiologists during

their studies of such phenomena. More complex behavior, particularly protein interactions such as antibody-antigen reactions, blood clotting, the agglutination of red cells by isohemagglutinins, and the activities of specific enzymes and hormones, involves more detailed molecular characteristics. Information concerning the molecular characteristics of the various plasma proteins yields (*1*) insight into the reactions with which the clinician must deal and (*2*) understanding of the properties the chemist must employ in the further development of the inclusive fractionation of plasma.

Many of the methods in use for the study of the physical chemical properties of proteins (1) involve the application of an external field of force and the study of the behavior of the various components of the system in an appropriate apparatus. The ultracentrifuge and the electrophoresis apparatus have been found helpful in studying mixtures of proteins, largely because of optical systems[4] which render it possible both to distinguish and to determine the relative amounts of the several components by their motion under the conditions imposed.

Measurements of diffusion, viscosity, double refraction of flow, osmotic pressure, electric moment, and the light-scattering properties of certain of the proteins in plasma have been made, but these studies are of great value only when applied to systems of one protein component, or at most, of fairly simple combinations of components. They contribute greatly, however, to our knowledge of the separated proteins.

ELECTROPHORESIS

If an external electric field of force is applied to protein molecules dissolved in a suitable solvent, it is found that in acid solutions, proteins move in one direction and in alkaline solutions, in the opposite direction. The significance of this phenomenon was recognized toward the end of the last century by the late Sir William Hardy. He and subsequent workers, notable among them Pauli and Michaelis, studied this amphoteric property of a variety of proteins. It remained for Tiselius,[5] however, to design the modern electrophoresis apparatus used in these studies.

Under a given field strength, the speed and direction of the movement of a protein will be determined by the pH, concentration and nature of the salts,[6] and the viscosity of the solvent. Two or more different protein molecular species in the same solvent will usually move with different velocities. This makes it possible to use these velocities as a means of distinguishing protein molecules differing in kind; the velocity in a unit electric field is defined as the electrophoretic mobility.

In this manner, analysis of plasma has yielded at least 6 electrophoretic components (2), as illustrated below in Figure 1. The fastest moving component in neutral or alkaline pH ranges is albumin. From the relative area of the peak which moves with this velocity, albumin is found to constitute about 55 per cent of the total plasma proteins.[7] Next in order of decreasing

[4] The optical systems have been devised and developed by Svedberg, Tiselius, Lamm, Philpot, Svensson, Longsworth, and others. In perhaps their most convenient form, the so-called Toepler schlieren (shadow) is employed to project a pattern on the photographic screen which can be resolved into a series of more or less skewed probability curves. The rate of motion is calculated from the change with time of the center of mass of each area. The area under each of such curves measures the concentration of the protein moving with that speed.

[5] By the use of a rectangular shaped cell with a maximum surface and minimum change of density when heated by the current generated by the electric field (near 4° C.), Tiselius was able to subject a protein solution to electrical gradients much larger than those previously used (up to 5 or 10 volts per cm., depending upon the buffer used) which permits the observation of a series of protein boundaries, moving through the solvent or solution with characteristic electrophoretic mobilities. The mobility, u, calculated from the rate of motion of each component, is generally expressed in cm. per second when the protein is in an electric field with a gradient of one volt per cm.

[6] The salts are usually selected to buffer at the desired pH. We have used potassium phosphate buffers of pH 7.7 and 0.2 ionic strength, or sodium diethylbarbiturate buffers of pH 8.5 and 0.1 ionic strength.

[7] The electrophoretic measurements have been made by M. J. E. Budka, A. H. Sparrow, and K. C. MacDonald, under the direction of S. H. Armstrong, Jr. The percentage composition of a protein solution derived from the areas under the peaks of schlieren diagrams, under certain conditions, represents the percentage of the total refractive increment (difference between the refractive indices of the solution and the solvent) contributed by each electrophoretic component (3). To convert this to weight percentage requires knowledge of the specific refractive increments of each component.

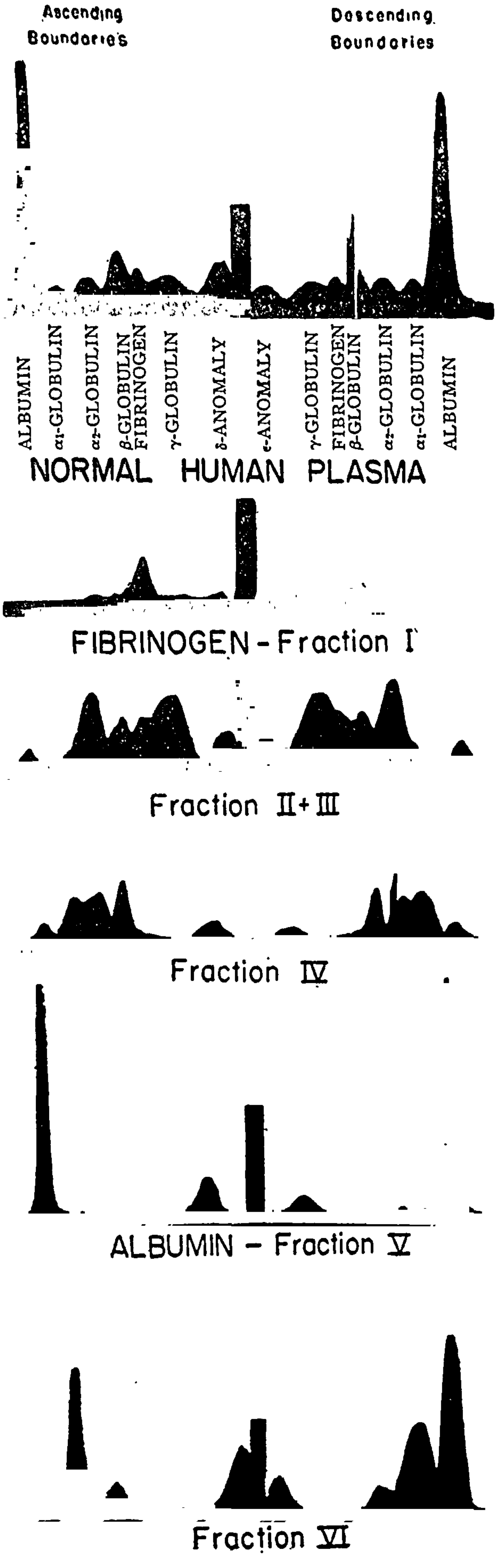

FIG. 1. ELECTROPHORETIC SCHLIEREN DIAGRAMS OF HUMAN PLASMA AND FRACTIONS I TO VI

velocity come the α-globulins, which under certain conditions divide into 2 separate components, designated as α_1 and α_2. Moving more slowly than these are the β-globulins, likewise sometimes separated into 2 components, β_1 and β_2. The total α- and β-globulins comprise about 13 and 14 per cent, respectively, of the total plasma proteins, as indicated by the areas of the separated peaks. Still slower moving is fibrinogen, which represents approximately 7 per cent of the plasma proteins. Slowest of all are the γ-globulins, estimated as representing 11 per cent of the plasma proteins.[8]

The electrophoretic components into which the Tiselius apparatus resolves the plasma proteins do not necessarily represent homogeneous molecular species, either with respect to size, shape, charge distribution, solubility characteristics, or physiological functions. The great advantage of the apparatus lies in its speed and simplicity of operation and in the reproducibility of the results.

SEDIMENTATION

When a protein solution is placed in an intense centrifugal field,[9] the molecules are observed to

[8] The almost stationary peaks, known as the delta and epsilon boundaries are nearly always observed in electrophoretic diagrams. It has been shown, however, that these peaks are not due to a protein component of plasma but to electrolyte concentration gradients (4). The discrepancy between the value for the fibrinogen content of the plasma proteins as obtained by electrophoretic analysis and by the nitrogen analysis of the washed clots is discussed in Paper XV of this series (5).

[9] During the last 18 years, Svedberg has developed the velocity ultracentrifuge for the measurement of the sedimentation rate in an intense centrifugal field (now as high as 400,000 times gravity) of molecules of the size of most proteins. This most important tool, the various improvements it has undergone, and many of the results that have been obtained with it, as well as the theory employed for the calculation of molecular weights from ultracentrifugal measurements, have been quite completely reported in a recent monograph by Svedberg and Pedersen (6). At its present stage of development, the centrifugal force developed is limited only by the strength of the alloys available for construction of the rotor. Recently, work of Beams, Bauer, Wykcoff, Pickels, and others have made the ultracentrifuge available to a somewhat larger number of

move with various rates of sedimentation. These rates, usually expressed and defined as the rates in a unit centrifugal field and called the sedimentation constants, vary almost directly with the viscosity and the difference in density between the protein and the solvent, but not in general to any large extent with pH and concentration.

The air-driven centrifuge designed by Bauer and Pickels has been used for studies of sedimentation in our laboratory,[10] while the oil-driven ultracentrifuge designed by T. Svedberg has been used by J. W. Williams at the University of Wisconsin for studies of many of these products (7).

Plasma or serum has been shown to contain a considerable number of components of different rates of sedimentation. Although undiluted plasma shows a more complex diagram, in dilute solutions, the main component is found to have a sedimentation constant near 4.6 S, characteristic of albumin. Fibrinogen, γ-globulin, and a part of the α- and β-globulins, have sedimentation constants near 7 S. In addition, there are small amounts of globulins sedimenting with constants between 7 and 18 S; and, although the results are not yet conclusive, it would appear that at least a part of the α- and β-globulins sediment, under certain conditions, with a rate close to that of albumin. Sedimentation constants of some of the constituents of plasma are listed in Table I.

Another important use of the ultracentrifuge is in studying protein denaturation. Proteins subjected to certain treatments, such as heating, become irreversibly altered from their native state, and sufficiently drastic treatment renders them insoluble in water and neutral salt solutions. Before properties of the proteins are changed greatly, it is often possible to detect preliminary subtle changes in the ultracentrifuge. Thus, solutions normally exhibiting a single protein component may show a number of components after being subjected to drastic treatment. We have accordingly established standards, making use of the ultracentrifuge, to detect changes in protein products, both during preparation and during storage.

workers by the development of a less complex air-driven machine.

The great strides made possible by this development depend in no small part upon the optical systems already mentioned which render it possible to distinguish more than one boundary and therefore components of more than one sedimentation rate. The sedimentation rates are usually expressed in terms of the rate in a unit centrifugal field, the quantity known as the sedimentation constant, s. Recorded values are usually expressed in Svedberg units (one Svedberg unit equals 10^{-13} cgs. units), corrected to a medium of the viscosity and density of pure water at 20° C., and designated $s_{20,w}$.

[10] The measurements have been made by C. G. Gordon and G. N. Thurber under the direction of J. L. Oncley.

OSMOTIC PRESSURE

Pfeffer and de Vries, while considering the forces necessary to draw water into tissues, made the pioneering studies on the passage of water through membranes which led Van't Hoff to the development of the concept of osmotic pressure. This phenomenon, extensively studied in the last century in terms of the rupture of plant and red blood cells, remains the most important factor in connection with the distribution of water, the membranes surrounding plant and animal cells being permeable to water and to many smaller molecules, but impermeable to the larger proteins which make up the greater part of the constituents of such cells. The pressure difference across such membranes at equilibrium measures the osmotic pressure, sometimes termed the colloid osmotic pressure or more recently the oncotic pressure.

Quantitative measurements of osmotic pressure at low protein concentrations can be used as a means of estimating the size of protein molecules, since the osmotic pressure depends as a first approximation upon the number of molecules or ions in a given volume of solution. The osmotic pressures of serum albumin solutions have been accurately measured and are considered in Paper VI of this series (9). A molecular weight of 69,000 has been estimated for this protein. Preliminary values for the colloid osmotic pressure of γ-globulin solutions have also been obtained. The osmotic pressure-concentration ratio for this protein varies much less with increasing concentration than is the case for albumin, and the molecular weight is in the neighborhood of 160,000 (see Table I).

In more concentrated solutions, the osmotic pressure depends to a considerable extent upon

TABLE I

Dimensions and physical constants of plasma proteins and other blood constituents

	Sedimentation constant $s_{20,w}$	Diffusion constant $D_{20,w} \times 10^{4}$	Viscosity coefficient F	Osmotic pressure-concentration ratio	Electrophoretic mobility pH 7.7 Γ/2 0.2	Molecular weight	Approximate dimensions (Ångstroms)		Negative net charge per molecule pH 7.4	Electric moment per molecule (Debye Units)
							Length of ellipsoid	Equatorial diameter of ellipsoid		
Sodium ion		139.[a]		6,000.[b]		23.0	1.9[c]	1.9[c]	−1	
Chloride ion						35.5	3.6[c]	3.6[c]	1	
Glucose		64.[a]	4.[a]	1,000.[a]	0	180.	9.5[d]	6.5[d]	0	
Serum albumin	4.6[e]	6.1[e]	5.8[f]	2.7[g]	5.3[h]	69,000.[i]	150.[j]	38.[j]	18[f,k]	400[m]
Serum γ-globulin	7.1[n]	3.8[n]	8.5[f]	1.2[f]	0.9[h,p]	156,000.[n]	320.[q]	36.[q]	8[k,r]	1,200[r,s]
Fibrinogen	7.[e]		70.[f]		1.9[h,p]	500,000.[t]	900.[u]	33.[u]		
Red blood cells			[v]			[w]	24,000.[x]	86,000.[x]		

a. From International Critical Tables, McGraw Hill, New York, 1929.
b. Calculated from molecular weight and osmotic coefficient.
c. These are the diameters of the sodium and chloride ion, as given by Pauling, L., "The Nature of the Chemical Bond," 2nd Edition, Cornell University Press, Ithaca, New York, 1940.
d. Calculated from dimensions of glucose models. See J. Phys. Chem., 1941, 45, 776.
e. Unpublished measurements of Oncley, J. L.
f. Unpublished measurements of Scatchard, G., and Brown, A., and Batchelder, A. C.
g. Scatchard, G., Brown, A., and Batchelder, A. C., Paper VI of this series (9).
h. Unpublished measurements of Armstrong, S. H., Jr.
i. Calculated from sedimentation and diffusion constants, and from osmotic pressure measurements.
j. Computed for an axial ratio of 4 and a hydration of 0.2 gram of water per gram of protein (8).
k. Unpublished electromotive force measurements of Cohn, E. J., Strong, L. E., and Blanchard, M. H.
m. Unpublished measurements of Oncley, J. L., and Gross, P., Jr.
n. The sedimentation and diffusion constants recorded here are those quoted by Svedberg and Pedersen (6) for total, electrophoretically isolated, human γ-globulin. There are indications that this sedimentation constant and the molecular weight, given as 176,000, are somewhat larger than those for our γ-globulin Fraction II, which represents only a part of the total γ-globulin of plasma, and probably more of the pseudoglobulin than of the euglobulin. Complete ultracentrifuge and diffusion studies have not yet been carried out on this subfraction, but a value of 156,000 for the molecular weight of such a preparation has been obtained from osmotic pressure measurements of Scatchard, G., Brown, A., and Batchelder, A. C. This value may well vary with the euglobulin/pseudoglobulin ratio of the γ-globulin fraction, lower values being characteristic of pseudoglobulin.
p. Luetscher, J. A., Jr., Electrophoretic analysis of the proteins of plasma and serous effusions. J. Clin. Invest., 1941, 20, 99.
q. Computed for an axial ratio of 8 and a hydration of 0.5 gram of water per gram of protein. Oncley, J. L., The investigation of proteins by dielectric measurements. Chem. Rev., 1942, 30, 433.
r. Measurements on horse-globulin were used to estimate this value.
s. Oncley, J. L., Electric moments and relaxation times of protein molecules. J. Phys. Chem., 1940, 44, 1103.
t. Estimated from measurements of sedimentation constant, viscosity, and double refraction of flow, the latter by J. T. Edsall and I. H. Scheinberg.
u. Computed for an axial ratio of 30.
v. Estimated from shape to be about 4.
w. A value of about 6,000,000,000,000 is obtained for the weight of Avagadro's number of red blood cells, which shows them to be about a billion times the weight of a serum albumin molecule.
x. These values are the maximum thickness and the equatorial diameter of the red blood cell in plasma as recorded by Ponder, E., The Mammalian Red Cell and the Properties of Haemolytic Systems. Gebrüder Borntraeger, Berlin, 1934.

properties of the protein other than size. Molecular properties of most interest in this case are the number and the distribution of the electric charges the protein bears. Measurements of both osmotic pressure and distribution of chloride ion across the membrane as the magnitude of the charge varies with change in pH and salt concentration reflect forces of physical chemical and physiological significance, also considered in Paper VI of this series (9).

DIFFUSION

Molecules of a solution originally confined to a given space will uniformly disperse in the course of time throughout a larger volume. Measurements of the rate of transfer of molecules across a given area under specific conditions [11] yield a diffusion constant, D, characteristic of the molecules. Protein molecules have small, and simpler molecules like sodium chloride and glucose have large values for this constant, as shown in Table I. The slow diffusion of proteins through certain animal membranes was one of the early observations which led to their classification as colloids by Thomas Graham and others. Although diffusion can be used for an approximate estimate of the size of the molecules, we have used the equations of Svedberg which include both sedimentation and diffusion constants for the calculation of molecular weights. These calculations also yield the deviation in hydrodynamical behavior of the actual molecule from a spherical particle of the same weight, and this makes possible an estimate of the shape and hydration of the molecules. The relations used were either in the form of the original equations, tables, nomograms, or graphs (6, 11, 12).

VISCOSITY

Resistance to the flow of fluids through capillaries is caused almost entirely by the viscosity of the fluid involved. Indeed, it was the physiologist Jean Poiseuille who, in order to learn more about the flow of blood, made the first precise measurements of viscosity and discovered the laws of flow through capillary tubes. The viscosity of a protein solution is influenced far more by the shape of the molecules than by their size. The simple theory demands that the viscosity of solutions of spherical, incompressible, uncharged molecules be the same, regardless of their size, provided they occupy the same volume fraction. Solutions of fibrinogen and the globulins are more viscous than those of albumins, not because the molecules are larger, but because they are more asymmetrical. Thus, the viscosity of blood is of the same order as twice concentrated plasma, a 25 per cent albumin, a 15 per cent γ-globulin, or a 2 per cent fibrinogen solution. The importance of this observation in the preparation of blood substitutes which will offer a minimum of resistance to flow is immediately apparent.

In dilute solutions, the increase of relative viscosity is proportional to the volume concentration of dissolved substance, the constant of proportionality being the viscosity coefficient, F. Measurements of F for certain plasma proteins are recorded in Table I and have led to estimates of the shape and solvation of the molecules [12] (11, 12).

DOUBLE REFRACTION OF FLOW

When a solution containing molecules of long rod-like shape is caused to move in such a manner that velocity gradients are set up, orientation of the elongated molecules will occur as logs are lined up due to the variations of velocity in different parts of a fast flowing stream. A beam of polarized light passed through such a solution will usually behave in the manner observed for birefringent crystals such as Iceland spar. This double refraction of flow, or streaming birefringence, can be quantitatively evaluated by proper measurements. It is particularly pronounced in solutions containing fibrinogen, indicating that fibrinogen molecules are many times longer than they are broad. Studies in our laboratory [13] have indicated a length in the neighborhood of 900 Ångstroms for fibrinogen, a length 6 times that of the albumins, the most symmetrical of the plasma proteins.

CHARGE DISTRIBUTION AND NET CHARGE

Considerations of size and shape alone do not yield complete characterization of proteins from

[11] Diffusion measurements have been made in this laboratory by J. L. Oncley. We have used the diffusion cell designed by Lamm (10).

[12] In the case of viscosity, we have a choice of several calculations for the relationship between viscosity coefficient and asymmetry. The equations of Simha (13) have given results in better agreement with other methods when protein solutions are involved. Values computed from this equation are tabulated by Mehl, Oncley, and Simha (14). For a recent review of viscosity measurements of substances of high molecular weight see Lauffer (15).

[13] We are indebted to J. T. Edsall and I. H. Scheinberg for these preliminary values made with an apparatus which will be described elsewhere and which reaches velocity gradients as high as 30,000 cm. per second per cm. (8).

the standpoint of chemical behavior nor of physiological function. Thus, a hydrocarbon chain of the same size and shape as albumin will stand in obvious contrast to the latter in low solubility in water or dilute salt solutions, in base-binding capacity, and in effective colloid osmotic pressure, as well as in nutritive function.

A plasma protein molecule may be considered as an array of acidic and basic groups of various spatial relations and strengths. These groups, when ionized, are the principal sites of electrical charge. The degree of ionization varies according to the chemical environment of the molecule and the position of the group in the molecule.

At blood pH, plasma proteins bear a negative charge, being present largely as sodium salts. This negative charge is due to the dissociation of free carboxyl groups of the dicarboxylic amino acids.[14] At strongly acid reactions, the proteins carry a positive charge, for under these conditions the carboxyl groups are not ionized and the positive charge is entirely due to ionization of the free groups of the basic amino acids.

Between blood pH, where the molecule is an anion, and an acid pH, where it is a cation, there is an intermediate reaction, the isoelectric point, at which the molecule behaves as though uncharged in an electric field. Nonetheless, the molecule under these circumstances contains large and equal numbers of positively and negatively charged groups.

The symmetry of distribution of groups capable of carrying a charge is of great importance for the behavior of a large molecule, for even though the net charge be zero, if a great many groups bearing charges of similar sign are closely crowded together at one portion of the molecular surface, they will give rise to an appreciable electrical field of force which can act both on other large charged molecules and on smaller electrolytes of the environment. If, however, positively and negatively charged groups are evenly distributed, the proximity of positive to negative charges will tend to cancel their electrostatic fields, and thus interaction with other charged molecules will be small. The distribution of these groups, and thus the electrical symmetry of the molecule, is expressed in terms of an overall electric moment.[15]

Of the plasma proteins thus far studied, the γ-globulins have the most asymmetric charge distribution and thus have strong interactions with other proteins and electrolytes. By contrast, the albumins have a very symmetric electrical structure. Although they possess nearly 100 negatively and 100 positively charged groups per molecule in the isoelectric condition, these are spaced so as to produce a small electric moment (Table I) and albumins interact weakly with other proteins and electrolytes.

The net charge of a protein molecule determines to a great extent the rate of its migration in an electrical field. It cannot be quantitatively evaluated from this rate however for, in electrophoretic measurements, there enter many factors not at present[16] susceptible of analysis. However, electrometric titration curves of proteins, yielding data on acid- and base-binding capacities, measure this net charge and the number of positively and negatively charged groups on the molecule at reactions over the isoelectric and physiological ranges of pH.

The amphoteric behavior of proteins as revealed in electrometric titration curves is a factor in many physiological reactions; it is by reason of the base-binding capacity that the proteins account for 16 of the 155 m.eq. of anions per liter of normal plasma. Although the most important mechanism for maintenance of blood pH is the carbonic acid-bicarbonate buffer

[14] At pH more alkaline than blood, the negative charge is further increased by dissociation of the phenolic hydroxyl and sulfhydryl groups. For a discussion of the amino acid composition of the plasma proteins, see Brand (16).

[15] The electric moment of a molecule with two equal and opposite charges, e, separated by a distance, d, is $\mu = de$. The electric moment of a more complex set of charges can be computed by calculating such an electric moment for each pair of charges and then obtaining a vector sum of these moments. Electric moments are usually expressed in Debye units, equal to 10^{-18} cgs. units.

[16] In measurement of electrophoretic mobility, the electrostatic forces between the charged molecule and the ions of the surrounding electrolyte give rise to the formation of an atmosphere of ions around the molecule. This ionic atmosphere alters the strength of the electrical field in the immediate vicinity of the protein molecule and increases the force resisting the motion. For a theoretical discussion see chapter by Hans Mueller in Cohn and Edsall (1).

system, a secondary buffering is provided by the plasma proteins.

The rôle of this secondary system may be quantitatively illustrated as follows:[17] If, in the plasma electrolyte distribution, the proteins are replaced by their equivalent amount of chloride and the carbonic acid concentration of the system maintained constant, the addition of about 16 m.eq. of fixed acid will result in a drop of pH from the physiological mean of 7.4 to 7.0. The presence of the plasma proteins in normal concentration permits the addition of 3 more m.eq. of fixed acid to attain the same drop. Approximately 75 per cent of the additional buffering power is contributed by the albumins which, gram for gram, have about twice that of the globulins in this pH range.

The high buffering power of the albumins is reflected in the fact that they possess a net charge of 18 at blood pH, the highest of the plasma proteins (Table I). The significance of high net charge (taken in conjunction with low molecular weight and high molecular symmetry from the standpoint of both shape and charge distribution) in fitting albumin for its physiological rôle in the osmotic activity of plasma has previously been discussed (17 to 20) and is considered in earlier studies and in subsequent papers of this series.

THE CHARACTERIZATION OF THE PLASMA FRACTIONS

Knowledge of the physical properties of the various plasma proteins has been an invaluable aid in so dividing plasma that proteins responsible for a particular function have been concentrated and can be used appropriately in the therapy of specific conditions. Our goal has been not the preparation of a limited number of products to the exclusion of others, but the development of an inclusive method by which all components of plasma may be preserved and made available in useful forms. In developing our system of plasma fractionation, two criteria have been followed as guides to the success of separations: (*1*) each fraction should be as homogeneous as possible, and (*2*) the more labile components should be preserved.

The criterion that each fraction shall approximate homogeneity leads to a series of fractions, each possessing a different specific function or functions connected with chemically discrete protein molecules. The criterion that the more labile components are preserved throughout the fractionation not only demands that the methods be completely inclusive but also gives some assurance that subtle changes have not occurred in the more rugged components. Since many biological reactions are sensitive to small chemical changes that are difficult to detect by *in vitro* methods, it is of immense importance to know whether the isolated proteins have been irreversibly altered or maintained in their native state. In human therapy, it is always necessary to prove that any proteins foreign to the human body do not initiate undesirable reactions. If proteins from a human source, such as normal human blood plasma, are irreversibly altered during or subsequent to their separation, they must be considered to be foreign to the human body and only used with great caution.

Undoubtedly, dynamic equilibria exist within whole blood so that destruction and synthesis of its components go on continually. However, a purified protein solution for therapeutic use should possess stability of both its physical and chemical properties. It has been found in many cases that the proteins isolated from plasma are stable under conditions that seriously alter plasma. This fact may depend to a considerable extent on the presence of enzymes and reactive groups within plasma which are capable of acting upon protein molecules, otherwise inherently stable. In some cases, it has been found that careful separation of lipoid material from a product by precise fractionation enhances thermal stability greatly.

Clearly, a subdivision of all of the plasma into 6 fractions is not adequate in order to achieve the high concentration and separation of all of the useful components of plasma. Subfractionations of all our initial fractions have, therefore, been developed, and in the case of Fractions I, II + III, and IV it has been possible further to separate and concentrate components possessing specific physiological

[17] The calculation is arbitrary in the sense that it ignores the rôle of the phosphates and is inapplicable to whole blood in that the effect of hemoglobin is not considered.

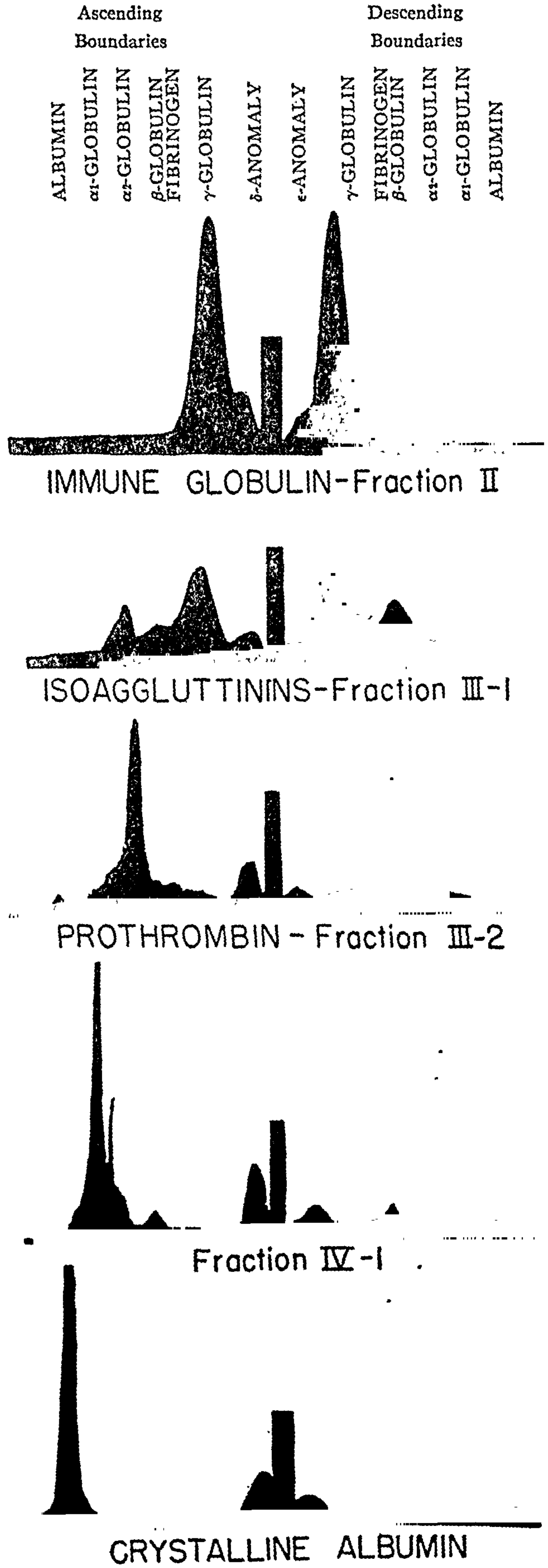

FIG. 2. ELECTROPHORETIC SCHLIEREN DIAGRAMS OF CERTAIN SUBFRACTIONS OF HUMAN PLASMA

activities (Figure 2). Moreover, it should be stressed that the smaller the fraction in which a component of therapeutic value can be concentrated, the more potent the activity of the concentrate, especially as compared to the plasma as a whole. The value of attaining the greatest possible concentration of each component, as well as its separation from other components, has emerged in the partial successes that have thus far been achieved.

Thus, in the case of antibodies, the higher their concentration in a fraction over that in plasma, the smaller the dose that may be necessary and, more important still, the more antibodies that can be injected in any practical dose. Thus, it is always possible that enough antibodies to give protection, or especially for treatment, could not conveniently be injected if the maximum concentration over the plasma that was achieved was 3- to 10-fold, whereas a 30- to 300-fold concentration of these specific antibodies might render them of clinical value. Moreover, certain active principles are present in such small amounts that they cannot be detected in plasma. The possibility that these human proteins have value for therapeutic use is opened when they are concentrated, and the useful limits of concentration cannot yet be envisioned.

The present state of development may be illustrated by the electrophoretic schlieren diagrams of the fractions in Figure 1. These diagrams should be compared with the diagram for whole plasma, whose analysis has been previously described. The diagrams, taken after electrophoresis has progressed to a comparable point in each case, are placed above each other so that proteins of like mobility in the plasma and in the fractions thereof should lie above each other in vertical lines. The peaks which have moved farthest from the central boundary are those of albumin. Albumin is largely concentrated in Fraction V, whereas the amounts in Fractions I, II + III, and IV are so small as to constitute a minor part of the total diagram. Likewise, Fraction II + III contains the larger part of the γ-globulin, which moves slowly. The quantitative information which can be obtained from these diagrams is given in Table II. Fraction I contains approximately 60 per cent of the fibrinogen in plasma; Fraction II + III contains

about 90 per cent of the γ-globulins; Fraction IV contains about 65 per cent of the α-globulins; and Fraction V contains about 85 per cent of the albumins.

The fractions upon which are based the clinical studies reported can also be characterized in terms of ultracentrifugal studies (7). Thus, the component of molecular weight comparable to the albumins is found in Fraction V. There is, however, a larger amount of material in the other fractions sedimenting with the velocity characteristic of albumin than can be accounted for on the basis of the amounts of albumin in these fractions, as estimated from electrophoretic analysis. Presumably, therefore, there is globulin in these fractions of smaller molecular weight than is characteristic of the bulk of the globulin, as has previously been claimed for some of the globulin in serum.

Conversely, the high molecular weight globulins have been largely concentrated in Fraction III–1, although a smaller amount has also been detected in other fractions. None is present in Fraction V.

PRODUCTS OF PLASMA FRACTIONATION

Proteins of interrelated functions do not always possess comparable chemical properties and are thus not always found in the same fraction. The products of plasma fractionation must also be considered, therefore, from the point of view of physiological function and clinical use. The largest categories are (*1*) the albumins, (*2*) the immune globulins, (*3*) the isohemagglutinins, (*4*) the hormones, enzymes, and related substances, and (*5*) the proteins concerned with the clotting of the blood.

The clotting of the blood depends upon a complex mechanism and upon a number of plasma proteins. Of these, fibrinogen, separated in Fraction I, may be considered the most important since its molecular properties determine to a large extent the mechanical properties of the clot (21). The conversion of a fibrinogen solution into the fibrin clot is brought about by the interaction of fibrinogen and thrombin. Prothrombin, rather than thrombin, is found in circulating plasma. Both are separated in Fraction III–2. The properties of these proteins, as well as of the other components that presumably play a rôle in clotting, such as the fibrinolytic enzyme and the hemophilic factor (both in Fraction II + III) are considered in Paper XV of this series (5).

The immune globulins are also found in Fraction II + III. These antibodies are largely concentrated in Fraction II, which consists in recent preparations of over 98 per cent γ-globulins as judged by electrophoretic analysis. The principal impurities consist of small amounts of β-globulin and albumin, of cholesterol and phospholipid. Like albumin, Fraction II can be dried and dispensed in the dry state. It has proved convenient, however, to make the immune globulins available in a concentrated solution. The solution is nearly colorless. In measles prophylaxis, only small amounts of this concentrated solution of antibodies are generally used. Immunological studies upon the anti-

TABLE II

Distribution of proteins of plasma[a]

	Grams protein per liter plasma	Grams protein per liter of plasma in fractions: I	II+III	IV	V	VI	Protein in fractions
Protein	60.3[b]	4.3	16.3	9.7	29.6	0.6	60.5
Albumin	33.2	0.2	0.7	1.0	29.0	0.3	31.2
α-globulin	8.4	0.2	1.8	5.4	0.6	0.3	8.4
β-globulin	7.8	0.8	6.2	3.1			10.1
γ-globulin	6.6	0.5	6.0	0.2			6.7
Fibrinogen	4.3	2.6	1.6[c]				4.2
Totals	60.3	4.3	16.3	9.7	29.6	0.6	60.6

a. The distribution of components in plasma and in each fraction is based on electrophoretic analysis which gives the fraction of the total refractive increment contributed by each component. The size of each fraction is based on nitrogen analysis, assuming a nitrogen factor of 6.25. It has been assumed that the refractive increment per gram of nitrogen is the same for all components. Work is in progress to determine the relations among nitrogen content, refractive increment, and dry weights for the various proteins.

b. For the total nitrogen of citrated plasma, an average value of 9.88 grams per liter has been taken. Of this, 9.65 grams per liter is protein nitrogen. Accordingly, the protein content of the plasma, which is obtained by multiplying by the conventional factor 6.25, is 60.3 grams per liter.

c. The electrophoretic analysis of Fraction II + III has been corrected to include 10 per cent of fibrinogen, precipitated as fibrin previous to analysis.

TABLE III

Products of human plasma fractionation

Components of plasma	Concentrated in plasma fraction	Percentage of plasma protein in fraction	Distribution of electrophoretic components in main fractions					Concentration ratio of principal electrophoretic component to that in plasma	Concentration of active function times that in plasma	Concentration of activity in final product times that in plasma
			Albumin	α	β	γ	Fibrinogen			
Albumin (Crystallized)			100	0	0	0	0	1.8	1.3[a]	5.4[b]
Albumin (Standard)	V	48	98.5	1.5	0	0	0[c]			
Hypertensinogen	IV	10	15	55	28	2	0	3.9	8[d]	
Complement C'2									8	
Complement C'1	III-2	3	0	10	75	15	0	5.8	15	
Thrombin									15[e]	20[f]
Isohemagglutinin	III-1	8	0	4	35	61	0	5.5	12	18[g]
Immune globulins	II	10	1	0	1	98	0[h]	8.8	8[j]	25[j]
Fibrinogen (Standard)	I	6	5	4	19	11	61	8.7		
Fibrinogen (Purified)			0	0	9	0	91	13		

a. Determined from osmotic pressure measurements. See Paper VI of this series (9).

b. Final product contains 250 grams of protein per liter.

c. Electrophoretic analyses are reported in Paper II (7).

d. Hypertensinogen activity estimated in cat units per gram.

e. Estimated in prothrombin units.

f. Calculated as ratio of thrombin units per mgm. of protein in thrombin preparation to prothrombin units per mgm. of protein in the original plasma pool.

g. Estimated as an approximately 9 per cent protein solution.

h. Electrophoretic analysis of Fraction II, prepared by more recent methods and shown by clinical trial to contain measles antibody. The preparations made earlier contained about 85 per cent γ-globulin, 10 per cent β-globulin, less than 1 per cent α-globulin, and 5 per cent albumin. Values for individual preparations are given in Paper II (7).

j. Average of estimates of activity of several different antibodies as reported in Paper X of this series (22).

bodies of plasma are reported in Paper X of this series (22), and clinical studies on their use in the prevention and modification of measles in Papers XI and XII of this series (23, 24).

The isohemagglutinins are also separated quantitatively in Fraction II + III and are further concentrated in Fraction III-1. When group specific plasma is used as the starting material for the fractionation, the resulting concentrate of the isohemagglutinins makes an acceptible blood grouping material. Both anti-A and anti-B isohemagglutinins have been concentrated at the Harvard pilot plant in this way at the suggestion of and in collaboration with Col. Callender and Lt. Col. Kendrick at the Army Medical School (Papers XIII and XIV (25, 26). The anti-Rh globulin has also been concentrated in this fraction and reduced to the dry state from low titered Rh positive blood collected by Louis Diamond of the Children's Hospital, Boston. It is hoped that the development of these typing materials as a part of the plasma fractionation process will contribute to the program of making available whole blood and resuspended red cells.

Complement is made up of a series of components which together participate in certain immunological phenomena. They are less stable than the antibodies and present in but small amounts. Though destroyed in our earlier attempts at plasma fractionation, it has recently been possible, in collaboration with Lt. Louis Pillemer, to obtain in almost quantitative yield, both the midpiece of complement, C'1, and the end piece, C'2. As was to have been anticipated from earlier work with complement of animal origin (27, 28), these components are concentrated in quite different fractions, C'1 in

Fraction III–2, and C′2 in Fraction IV–2. Their use in physiology and medicine has not yet been established.

Enzymes and hormones serve many different functions, possess very different properties, and are found in different fractions. Thus, a fibrinolytic enzyme is largely concentrated in Fraction III–2, as is the globulin which promotes the clotting of hemophilic blood. Alkaline phosphatase is concentrated in Fraction IV. Whereas the concentration of enzymes from animal and vegetable sources has yielded products of great value and this may be expected also of the enzymes of human plasma, our efforts thus far have been concentrated on freeing the albumins and immune globulins from enzymes in the interest of high thermal stability.

The hormones found in the blood stream will presumably be present in varying amount from time to time and have their origin in many cases in the various glands of the body. It has not been possible to prove thus far that the hormone, concentrated from a tissue extract, is identical to that concentrated in a fraction of the plasma, but it has been possible, in collaboration with F. L. Hisaw, to detect, in plasma fractions, the presence of luteinizing, follicle stimulating, and thyrotropic hormones. The 2 gonadotropic hormones have been found in only certain plasma pools, the luteinizing in Fraction II + III, the follicle stimulating hormone in Fraction VI. The thyrotropic hormone appears to be a more constant component of our fractions where it is concentrated in Fraction IV–3, 4.

The protein-bound iodine of plasma, for which assay has been made by W. T. Salter, has also been largely found in Fraction IV, some also in Fraction V. The investigation of these proteins is continuing.

Hypertensinogen has been concentrated in Fraction IV–2, 3. Special problems, however, have been encountered in obtaining a satisfactory stable hypertensinogen concentrate. We are indebted to Lewis Dexter for assay for this blood pressure regulating substance.

The lipo-proteins are largely concentrated in Fractions III and IV and appear to be associated with α- and β-globulins. Indeed Fraction I, rich in fibrinogen, Fraction II in the immune globulins, and Fraction V in albumin have been prepared with such low content of lipid material as to suggest that its presence represents impurities in these fractions.

Although detailed investigations are in progress [18] of the nature of the lipid material which has been concentrated largely in Fractions III–1, IV–1, and IV–4, distribution has thus far been followed by the determination of cholesterol, carotene,[19] and phospholipid. Approximately two-thirds of the total plasma cholesterol separates in Fraction II + III where it appears to be associated with the β-globulins with which it is further concentrated in Fraction III–1. Over half of the carotene and phospholipids are also concentrated in Fraction II + III.

The albumin of plasma represents by far its largest component and is concentrated therefore in its largest fraction, V. Whereas there is evidence of the existence of more than one albumin, this fraction appears homogeneous in the ultracentrifuge and in the electrophoretic apparatus at neutral or slightly alkaline reactions.[20] The standard of purity specified in the Navy contracts for the production of this blood substitute permits 2 per cent of globulin. Electrophoretic analyses, reported in Paper II of this series (7), reveal the constancy of the product under the conditions of industrial production.

Human serum albumin has been still further purified by crystallization, and study of the crystallized albumin that we have prepared has demonstrated that the very high thermal stability and low viscosity of our standard preparations are indeed ascribable to the albumin, instability largely to globulin and lipid impurities. Comparison of thermal stabilities of crystallized and of standard normal human serum albumin is considered in Paper IV of this series

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[18] Large amounts of Fraction IV-1 have been made available to A. Chanutin and H. E. Carter for these investigations which will subsequently be reported elsewhere. The distribution of these by-products of plasma fractionation for chemical studies has been directed by H. B. Vickery.

[19] We are indebted to N. Talbot for carrying out assays for carotene and cholesterol in our earlier fractions, assays which are now being continued and expanded by P. Gross.

[20] Conditions under which electrophoretic separations among the albumins have been effected are not considered at this time, though the studies of Luetscher (29) have been extended by S. H. Armstrong, Jr., and M. J. E. Budka.

(30), the electrolytes which appear to contribute the most favorable environment for the concentrated albumin solutions in Papers IV (30) and V (31) of this series. Normal human serum albumin can be prepared in far larger amounts and with higher yields if further purification by crystallization is not superimposed upon the process. The standards of purity determined upon for the albumin now being delivered to the Navy in large amounts were chosen so as to assure freedom from untoward reactions with maximum efficiency in large scale production.

Serum albumin was developed in order to attain a blood substitute which could be distributed in solution ready for immediate emergency use. It has been made available as a 25 per cent solution to render the package as compact [21] as possible for transport. Osmotically 4 times as concentrated as plasma, 25 per cent albumin is no more viscous than whole blood. Both the advantages and the contraindications to the use of this hypertonic solution are considered in other papers, both from the point of view of theory and of practice.

The properties and uses of albumin are considered in Papers II to IX (7, 9, 16, 30 to 34); those of the immune globulins in Papers X to XII (22 to 24); those of isohemagglutinins in Papers XIII and XIV (25, 26); while the properties and uses of the various products that have been prepared from the clotting proteins are considered in Papers XV to XXII (5, 21, 35 to 40) of this series.

The development and interrelations of these different products has been our special concern. The physical chemical characterization of the protein products which are given in this paper are supplemented by their amino acid analyses in Paper III of this series (16). Wide differences in the nature of the specific chemical groups upon which the fine structure and specificity of behavior of these proteins presumably depend are revealed in this preliminary report. Analytical knowledge of this kind was sought in the interest not only of chemical control but also of determining the significance of the various fractions from the point of view of nutrition, especially of plasma and blood regeneration, considered thus far only in a single preliminary communication (Paper XXIII of this series) (41).

As each of these products is developed at the Harvard Plasma Fractionation Laboratory, sufficient amounts are prepared in our pilot plant [22] to permit investigation of conditions for its distribution in a sterile [23] and stable state and of the reproducibility of the preparative process and of its clinical appraisal under diverse conditions.

All the products of plasma fractionation that have thus far been recommended to the armed forces have been reduced to the dry state in their preparation. In the dry state, stability may be expected to be maximal, whereas stability in solution will vary from product to product and depend also upon the properties of the solvent. The final form for a given product has thus been decided following consideration both of its maximal stability and of medical and military requirements.

SUMMARY

Plasma has been subjected to a large scale fractionation process which yields the different proteins of which it is composed, separated and concentrated. Each concentrate can then be used in therapy in relation to its specialized natural properties and functions.

The physical dimensions and properties of the plasma proteins differ widely. All that have been studied, however, have equatorial diameters close to 36 Å, a dimension which prevents their rapid loss from the blood stream.

The plasma proteins, however, vary in length from 150 Å, characteristic of serum albumin, to 900 Å, characteristic of fibrinogen. It follows that solutions of the more symmetrical albumin

[21] The package adopted by the armed forces for the standard 25 per cent albumin solution was developed by Capt. Lloyd R. Newhouser, Medical Corps, United States Navy, and Lt. Col. Douglas Kendrick, Medical Corps, United States Army.

[22] The continuous operation of our pilot plant for two and a half years has made possible the preparation of each product developed and its standardization for clinical trial. The staff of the pilot plant have included: L. E. Strong, W. L. Hughes, Jr., D. J. Mulford, M. Melin, J. N. Ashworth, J. H. Cameron, H. T. Gordon, H. L. Taylor, D. A. Richert, R. W. Kelty, L. H. Larsen.

[23] We are indebted to the Antitoxin and Vaccine Laboratory, Massachusetts Department of Public Health, for its collaboration in preparing for distribution in sterile and safe form both normal human serum albumin and the immune serum globulins which are now licensed products under the National Institute of Health.

molecules are far less viscous than are those of other plasma proteins.

The molecular weights of the albumins being less than half as great as those of most globulins, their osmotic activity is more than twice as great. Albumins have large numbers of charged groups, symmetrically arranged, and a large net charge which also results in increased osmotic activity.

The immune globulins have a smaller net charge, but far greater electric moments, rendering them more reactive with other proteins and electrolytes.

The size and shape of the molecules and the nature and structural interrelations of their free groups determine the physico-chemical characteristics and presumably the physiological functions of the plasma proteins. The plasma proteins differ in their solubility and in their stability in solutions of different pH, temperature, dielectric constant, and concentration of dipolar ions, electrolytes or molecules rich in hydroxyl or other groups. The methods of separation that have been developed depend upon these differences in physico-chemical properties.

The products that have thus far been made available as stable, dry white powders in sufficiently large amounts for clinical appraisal include: (*1*) *fibrinogen*, upon which the physical properties of the blood clot largely depend, which can be made into plastics, or in connection with (*2*) *thrombin* into (*3*) *fibrin films*, used as membranes and (*4*) into *fibrin foams*, used as hemostatic agents in neurosurgery; (*5*) the *immune globulins* proven of value in measles prophylaxis; (*6*) the *isohemagglutinins* in blood typing; and (*7*) *albumin*, which represents less than 60 per cent of the plasma protein but is responsible for approximately 80 per cent of the osmotic activity of the blood and is used in the treatment of shock.

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more recent electrophoretic studies have been carried out at a more alkaline pH and a lower ionic strength. A diethyl barbiturate buffer, first introduced by Longsworth (2), of pH 8.5 and 0.1 ionic strength has been used. This buffer gives a more complete separation of α-globulin and reveals somewhat more total globulin, since much of the α-globulin is not distinguished from albumin when studied in the phosphate buffer at pH 7.7. Such analyses are summarized in Table II. It will be seen from these results that only small amounts of α-globulin are detected in these serum albumin solutions (average of 1.5 per cent) and that fibrinogen, β-, and γ-globulin are all absent within the limits of sensitivity of the test.

Ultracentrifugal analyses of serum albumin solutions have not been routinely carried out, since the sedimentation diagrams depend to a considerable extent upon the ionic strength and pH of the solvent. Preparations of serum albumin thus far studied reveal slightly asymmetrical sedimentation diagrams in the ultracentrifuge. Whatever the interpretation of this asymmetry, it is clear that it must be due to a small amount of material with a sedimentation constant very nearly that of normal serum albumin and too small to be mistaken for that characteristic of normal serum globulin. Such diagrams have indicated, however, that materials of a sedimentation constant, very different from normal albumin, are not present within the accuracy of this method of analysis. Since it has been demonstrated that faster sedimenting components are sometimes formed as a result of drastic conditions introduced in the fractionation of certain unstable albumin preparations, it is of considerable importance to have demonstrated the absence of such material in all other preparations of albumin that have been studied. As a routine test, such unstable preparations have been more readily detected by nephelometric and viscometric stability studies, reported by Scatchard and coworkers, in the fourth paper of this series (3).

TABLE II

Electrophoretic analysis of normal human serum albumin preparations. Average distribution of components

(Barbiturate or veronal buffers pH 8.5, ionic strength 0.1)

Processing plant	Number of analyses	α_1 Globulin	α_2 Globulin	β Globulin	Albumin
B	12	0.7	0.7	0	98.6
C	8	1.2	0.9	0	97.9
D	9	0.9	0.8	0	98.3
E	4	0.5	0.6	0	98.9
F	9	0.7	0.6	0	98.7
G	20	1.4	0.8	0	97.8
Total	62				
Average		0.9	0.7	0	98.4

HUMAN IMMUNE SERUM GLOBULIN

Electrophoretic analyses of nearly all of the preparations of immune globulin have been carried out by using diethyl barbiturate buffer, pH 8.5, ionic strength 0.1. The separation of β-globulin from both α- and γ-globulin is not complete under these conditions, and the analyses therefore are somewhat unsatisfactory. They have, however, been carried out in the same way for all of these solutions, and the last 4 columns of Table III record the values obtained by a standardized procedure. It will be seen that some albumin and β-globulin are present in these preparations. If the results obtained with all preparations are averaged, we obtain values of about 2 per cent albumin and about 11 per cent β-globulin. The preparations fractionated by later methods (3A and 3B) average about 2 per cent albumin and 4 per cent β-globulin, whereas those fractionated by the more recent method (see Table III, Paper I) (1) consist of over 98 per cent γ globulin.

Ultracentrifugal analyses of all these solutions were carried out with 0.15 molar sodium chloride as solvent. The sedimentation studies were made at the pH of the immune globulin preparation, usually between pH 6.8 and 7.4. The ultracentrifuge components have been designated as slow moving, normal, and fast moving. The sedimentation constants of the slow moving components were of the order of 4 to 5 Svedberg units, and presumably represented albumin at least in large part.[5] The fast moving components had sedimentation constants varying from 8 to 18 Svedberg units, and this high molecular weight material must be largely γ-globulin since

[5] See the discussion of sedimentation (1).

TABLE III

Chemical and physicochemical assay of various preparations of Fraction II

Preparation number	Method of fractionation	Cholesterol concentration	Ultracentrifugal analysis			Electrophoretic analysis			
			Slow	Normal	Fast	Alb.	α-Glob.	β-Glob.	γ-Glob.
		mgm. per ml.							
A48	2	0.2	9	78	13	8	0	4	88
A54R	2	0.3	14	77	9	8	0	8	84
A54K	2	0.2	13	78	9	8	1	9	82
A58	2	0.1	10	81	9	9	0	12	79
A29	3*	0.6				4	1	6	89
D26	3*	0.6	8	78	14	4	0	13	83
A35	3*	1.2	4	80	16	2	1	8	89
A74B	3*	0.9	4	75	21	1	0	14	85
C36	3	0.7	4	78	18	2	0	13	85
A66	3	0.9	6	80	14	2	1	4	93
A72	3	0.5	4	82	14	2	0	16	82
C51	3	0.7	7	80	13	1	0	9	90
C70	3	0.7	5	78	17	2	0	17	80
C80	3	1.3	4	79	17	1	0	14	85
C97	3	0.5	4	77	19	1	0	8	91
C102	3	0.5	5	74	21	1	0	13	86
C103	3	1.1	4	73	23	1	0	12	87
C104	3	1.5	3	65	32	0	0	21	79
C105	3	2.2	3	74	23	0	0	17	83
C106	3	1.7	3	74	23	0	0	15	85
C107	3	1.9	2	74	24	1	0	11	88
C108	3	1.3	4	74	22	1	0	16	83
C109	3	1.3	3	78	19	1	1	13	85
D36	3	1.3	2	78	20	1	0	17	82
A80	3	1.3	3	75	22	1	0	21	78
A84	3	0.3	4	74	22	1	0	11	88
A109	3	0.9	3	68	29	2	0	13	85
A97	3A	1.0	5	70	25	3	1	6	90
B1	3A	0.2	2	78	20	2	0	2	96
B2	3A	0.2	5	71	24	3	0	2	95
A74A	3A*	0.2	3	72	25	1	0	4	95
AS84	3A*	0.2	6	75	19	4	1	4	91
A269	3B*	0.7	4	72	24	2	0	6	92
A291	3B*	0.5	6	69	25	1	0	5	94
A111	3B	0.4	5	74	21	1	0	4	95
Grand average		0.8	5	75	20	2	0.2	11	87
Average methods 3A and 3B		0.4	5	73	23	2	0.2	4	94

* These preparations were derived from Fraction II + Fraction III which had been frozen.

in amount it is often in excess of the amount of electrophoretically determined α- and β-globulins. Of this faster moving material, only a small percentage is material of sedimentation constant 18; the bulk of it represents material moving only slightly faster than normal globulin.

Cholesterol analyses are also reported in Table III.[6] The values recorded here are in milligrams per milliliter, and should be divided by 2 in order to express the percentage of cholesterol in this material, since these solutions all contain about 200 mgm. of protein per ml. It will be observed that these values have a considerable range, higher values usually being observed for preparations high in β-globulin by electrophoretic analysis. An average value of about 0.8 mgm. cholesterol per ml. is obtained from all preparations, the more recent methods (3A and 3B) yielding a lower average of about 0.5 and often as low as 0.2 mgm. per ml.

[6] These analyses have been carried out by Paul Gross at the Department of Physical Chemistry, Harvard Medical School, following the method of Bloor, Pelkan, and Allen (4).

SUMMARY

Results of electrophoretic and ultracentrifugal analyses on serum albumin solutions have indicated that fibrinogen, β-, and γ-globulin, and components of molecular weight as large or larger than the normal globulins of plasma, are not present within the accuracy of these methods of analysis. The electrophoretic analyses have been carried out on 162 preparations delivered to the armed forces by 7 different laboratories and indicate that the albumin is routinely concentrated by this method of fractionation from a value of 55 or 60 per cent in plasma to a value of 98.5 per cent.

The immune serum globulins of 35 preparations from 4 laboratories have been studied and indicate that the γ-globulin content of these materials has been increased from about 11 per cent in plasma to about 87 per cent, and, in most of the more recent preparations, to over 95 per cent, the main impurities being β-globulin and albumin. An average value of very nearly 20 per cent of fast moving material, in large part γ-globulin, has been observed in the ultracentrifuge, the amount of this material being quite uniform in nearly all preparations.

These studies have given evidence of the reproducibility of these materials, and have provided the chemical specification of purity used in setting up minimum requirements for the acceptance for the armed forces of these products of plasma fractionation.

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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

III. AMINO ACID COMPOSITION OF PLASMA PROTEINS[1]

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The determination of the proportions of the amino acids that are liberated when proteins are subjected to complete hydrolysis provides information of value from many points of view. Amino acids are the fundamental units from which the protein molecule is constructed since the basic pattern of the structure depends upon the combination of the α-carboxyl group of one amino acid with the α-amino group of its neighbor to form peptide linkages. Accordingly, the conclusion may be drawn that the accessory polar groups of the basic and dicarboxylic amino acids commonly found in proteins are for the most part either free or are available for chemical combinations of various degrees of stability. Such combinations may occur between two polar groups located sufficiently near together within the same molecule, or they may give rise to interaction between separate protein molecules, or between the protein molecule and the molecules or ions of substances likewise present in the solution, or between the protein molecule and the molecules or ions of the solvent itself.

The physical chemical analyses of the human plasma fractions which are set forth in Paper I of this series (1) treat them as more or less elongated ellipsoids. The surface of these molecules may be considered as containing numerous discrete points at which a wide variety of chemical reactions can occur. Thus, at the point at which the basic amino acid lysine enters into the structure, under ordinary physical conditions, the positively charged free amino group of this constituent will be present. At this point, therefore, combination or interaction with negatively charged ions in the solution is possible. At another point on the molecule, there may be present the negatively charged carboxyl group of glutamic or aspartic acid and here, accordingly, interaction with positive ions in the solution can occur. Distributed over the surface of the protein molecule are hydrocarbon groups such as those of valine, leucine, and phenylalanine, which are of a non-polar type and therefore do not readily interact either with ions or molecules in aqueous solution. At other points are groups of greater or less degrees of polarity, such as the hydroxyl groups of tyrosine, serine, and threonine, and the sulfur groups of cystine, cysteine, and methionine. With these, interactions of various types can occur. The properties of protein molecules are thus to a great extent functions of the relative proportions in which the several constituent amino acid radicals occur and of the pattern according to which these radicals are arranged or distributed in the surface of the molecule.

The complexities of this concept are such that interpretation of the properties of a protein in terms of its amino acid composition is at the present time possible only in a preliminary and tentative fashion. Nevertheless, some success has been achieved. Thus, the acid-combining power of a protein should be equal to the sum of the basic groups, and the base-binding power equal to the sum of the free acid groups. The first relationship does in fact hold fairly closely in a number of cases of which 3 are shown in

[1] The present analytical studies of plasma proteins have been carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Columbia University. The preparations examined were, with one or two exceptions, made from blood collected by the American Red Cross by the Department of Physical Chemistry, Harvard Medical School, Boston, Massachusetts, under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

TABLE I

Composition of serum albumin of man, cattle, and horse

	Human[1] No. 42	Bovine[2] No. 456	Horse B[3]
Assumed molecular weight[4]	70,000	70,000	70,000
Average residue weight	113.3	112.5	114.4
Approximate number of amino acids per mole[5]	618	620	612
Approximate number of peptide bonds per mole[6]	582	583	577
Approximate number of sub-units (polypeptide chains) per mole[7]	36	39	35
Approximate average size of sub-units	1,900	1,800	2,000
Approximate average number of amino acids per sub-unit	17	16	18
Total number of basic groups by analysis, per mole[8]	105	111	117
Total number of basic groups by acid combining power, per mole[9]	99	103	98
Ratio of basic amino acids to total number of amino acids[8]	1:9.0	1:8.6	1:7.5
Total number of hydroxy-amino acids, per mole[10]	73	89	85
Ratio of hydroxy-amino acids to total number of amino acids	1:8.4	1:7.0	1:7.2
Total number of sulfur atoms in S-S linkages per mole	32	32	30
Ratio of sulfur atoms in S-S linkages to total number of amino acids	1:19	1:19	1:20

	Per cent			Number of residues or atoms per mole		
	Human	Bovine	Horse B	Human	Bovine	Horse B
Total nitrogen	15.95	16.07	15.90	797	803	795
Free α-amino nitrogen	0.72	0.78	0.70	36	39	35
Amide NH_3	1.07	1.05	0.90	44	42	37
Arginine	6.15	6.20	5.50	25	25	22
Histidine[11]	3.52	3.83	4.31	16	17	20
Lysine	5.8	6.5	8.4	28	30	40
Total protein sulfur	1.96	1.94	1.78	42	42	39
Cysteine	0.70	1.10	1.13	4	6	7
Half-cystine	5.58	5.41	5.23	32	32	30
Methionine	1.28	0.81	0	6	4	0
Tryptophane	0.19	0.58	0.30	0.6	2	1
Tyrosine	4.66	5.49	4.66	18	21	18
Serine	3.7	4.5	4.8	25	30	32
Threonine	5.03	6.46	5.97	30	38	35
Hydroxyproline	0			0		
Leucine	11.9	13.7	10.1	64	73	54
Glycine[12]		1.97			18	
Aspartic acid[12]	9.9	10.35		52	54	
Glutamic acid[12]	17.1	16.9		81	80	

[1] Human serum albumin No. 42, a crystalline preparation.

[2] Bovine serum albumin No. 456, a crystalline preparation.

[3] Horse serum albumin B (carbohydrate free fraction, so designated by Kekwick, R. A.: Observations on the Crystallizable Albumin Fraction of Horse Serum, Biochem. J., 32, 552–562 (1938)) prepared by Mr. Manfred Mayer of the Department of Medicine, College of Physicians and Surgeons, according to the method of Adair, G. S., and Robinson, M. E.: The Specific Refraction Increments of Serum-Albumin and Serum-Globulin, Biochem. J., 24, 993–1011 (1930).

[4] The number of residues (or atoms) are calculated on this basis.

[5] For calculation see Brand, E., and Kassell, B. (reference 5 in text).

[6] Total number of amino acids less number of free α-amino nitrogen groups.

[7] Equal to the number of free α-amino nitrogen groups, cf. text.

[8] Basic groups are equal to the sum of the free α-amino nitrogen groups and of the basic amino acids (arginine, histidine, and lysine).

[9] Acid combining power: Human and bovine serum albumin from data obtained in the Department of Physical Chemistry, Harvard Medical School. Horse serum albumin: Value quoted from Proteins, Amino Acids, and Peptides, by Edwin J. Cohn and John T. Edsall, Reinhold Publishing Corp., New York, 1942, page 355.

Table I. The measurement of total base-binding power is, however, difficult, and the accurate determination of the free acid groups in proteins has been accomplished in only a few cases. However, it appears clear that, in the physiological pH range, the negative charges carried by a protein molecule arise from the ionized free carboxyl groups of dicarboxylic acid residues (and terminal carboxyl groups, if present). Positive charges arise from the guanidinium groups of the arginine, and the ϵ-ammonium groups of the lysine residues, all of which groups may be considered to be positively charged at reactions acid to pH 8. The imidazole groups of histidine, and any α-amino groups present in the molecule, carry positive charges when acid to pH 6, and are uncharged when alkaline to pH 8.5 or 9. It is in the physiological pH range that these groups give up, or take on, positive charge; and it is therefore these groups that are almost entirely responsible for the buffering power of proteins under physiological conditions.

All of the plasma proteins carry a net negative charge in this range. This net charge is small compared with the total number of charged groups on the molecule, referred to in the preceding paragraph, and is obviously equal to the algebraic difference between the sum of the total positive and the total negative charges.

Other correlations between amino acid composition and behavior exist, but, in the present state of the theory of protein structure, are less clearly defined. As an example may be mentioned the present-day view that the protein molecule is made up of what are apparently the equivalent of a group of polypeptide chains, often referred to as "sub-units." This hypothesis is an attempt to explain the analytical fact that many proteins contain more amino nitrogen than can be accounted for as the amino nitrogen of lysine radicals. It throws much light upon many other properties of proteins, however, such for example as certain phenomena that accompany denaturation, and upon the fact that many proteins can be obtained in the form of films or fibers. A discussion of this view has recently been given by Chibnall (2).

Information concerning the amino acid composition of the plasma proteins has special significance in medicine. The formation of the plasma proteins proceeds continuously within certain tissues of the body, among which the liver appears to play a prominent part (3). At the same time, the process of protein breakdown goes on; isotope studies, for instance, have indicated that the mean half lifetime of certain serum protein molecules in the body is of the order of two weeks (4). When the plasma protein content of the body is depleted, as from burns or hemorrhage, or in certain types of disease, extra demands for the regeneration of these proteins by the body must be met (3). It is therefore of importance to know as accurately as possible the amino acid composition of plasma proteins, in order to evaluate the nutritional problems involved in regenerating them when they are deficient; and such studies form an integral part of the general program which is set forth in this series of papers.

Conversely, the injection of whole blood, plasma, or plasma protein fractions to treat certain specific clinical conditions represents also the injection of materials which, in the course of time, are broken down in the body and used for other metabolic processes. These human proteins are not dealt with as foreign substances by the human organism, but become part of its structure. For this reason, also, it is important to know accurately the nature of the contribution which they make to the total chemical economy of the organism.

As distinctive chemical differences are detected between different plasma protein fractions, analytical methods may sometimes serve as a control and guide in the process of fractionation, as do the physico-chemical methods

[10] Hydroxy-amino acids: Tyrosine, serine, threonine, and hydroxyproline.

[11] The histidine values were obtained by a recently developed procedure (15) involving separation of histidine as mercury complex and subsequent colorimetric determination. Dr. H. B. Vickery (personal communication) has found 3.22 per cent of histidine in human and 3.46 per cent in bovine serum albumin by a new isolation procedure.

[12] The values for glycine, aspartic acid, and glutamic acid were obtained by Dr. David Shemin, under the direction of Professor H. T. Clarke of the College of Physicians and Surgeons, Columbia University, by the isotope dilution method. The preparations analyzed were crystallized bovine albumin lot 17 and crystallized human albumin (COM-1).

discussed in Paper I of this series (1). Analytical methods have already served as valuable tools in the solution of various problems that have arisen and should become even more significant in the future.

ANALYTICAL METHODS

The micro methods used for the determinations of the various amino acid constituents of the human plasma proteins that have been studied can be divided into 3 groups that yield results that are accurate: (*a*) within about 2 per cent, (*b*) within about 5 per cent, and (*c*) with errors possibly greater than 5 per cent. In the first group belong the values for total nitrogen (micro Dumas), total sulfur (Pregl, cf. (5)), amide nitrogen (6), arginine (7), cysteine (5, 8, 9), cystine (8, 9), methionine (9), tryptophane (10), tyrosine (11), threonine (modifications (12) of (13)), leucine (14), and histidine (15). To the second group belongs the determination of serine (modification of (16)), the serine values being corrected for 10 per cent destruction during acid hydrolysis (17). In the third group are the results for free α-amino nitrogen and for lysine, which were estimated from the rate of liberation of nitrogen in the manometric Van Slyke apparatus (15, cf. 2, 18). Hydroxyproline was not detected in human serum albumin (see Table I), when the colorimetric test of McFarlane and Guest (19) was applied.

The values for the average residue weights may be slightly too low; they are affected mainly by the uncertainty in the distribution of the free amino nitrogen of the protein between the true free α-amino nitrogen and the ϵ-amino nitrogen of lysine.

SERUM ALBUMIN

The composition of the serum albumin of man, of cattle, and of the horse is reported in Table I. The results are given in percentage by weight and in terms of residues per mole on the basis of an assumed molecular weight of 70,000, a figure from which the actual molecular weights of the 3 albumins differ only slightly (*cf.* 1). With proteins having the degree of homogenity apparently possessed by the samples discussed in this report, the computations in terms of residues per mole are of value because they make the comparison between different proteins easier and may ultimately be of help in the correlation of chemical composition with physico-chemical properties.

In the case of the bovine albumin, about 85 grams of the split products per 100 grams of protein are identified and 430 out of 620 amino acids accounted for. For human albumin the data in Table I show 77 grams of the split products, and 381 out of 618 amino acids.

The data show that there are considerable differences in the amino acid make-up of these 3 blood proteins, although in a general way human and bovine albumin resemble each other fairly closely and differ from that of the horse. The complete absence of methionine, an essential amino acid, from horse serum albumin-B is especially noteworthy. The carbohydrate-containing horse serum albumin-A likewise contains no methionine (12). This is of interest in view of the claim of Whipple and his coworkers (20) that cystine is an essential sulfur amino acid for serum protein regeneration (in dogs) while methionine is ineffective.

The tryptophane content of all 3 albumins is low, about 1, 2, and ½ a residue per mole, respectively, in horse, bovine, and human serum albumin. The fractional value for tryptophane, which has repeatedly been obtained in different highly purified preparations of human albumin, suggests that these preparations are still not chemically homogeneous.

The hydroxy-amino acids (tyrosine, serine, and threonine) account for about 12 to 14 per cent of the weight of these proteins. Although high, this is by no means unusual. The similar high hydroxy-amino acid content of β-lactoglobulin (5, 12) may be mentioned as an example, and it would appear that about 1 out of 8 of the constituent amino acids of the serum albumins is a hydroxy-amino acid.

The number of residues per mole of cysteine, leucine, and lysine in the 3 albumins shows some variability, while the content of arginine and of histidine is fairly uniform. The present evidence suggests that the lysine content of horse serum albumin is unusually high.

The dicarboxylic amino acids account for about 27 per cent of the weight of both human and bovine serum albumin. The distribution between glutamic and aspartic acid is practically identical in these 2 proteins (*cf.* Dr. Shemin's data in Table I).

The uniformity in the free α-amino nitrogen in the 3 albumins is striking as well as the total number of basic groups and the number of disulfide linkages. The molecule of serum albumin seems to be characterized by about 16

TABLE II

Composition of human γ-globulin

Preparation No. 36, II-1 (H)[1]

Assumed molecular weight[2]	171,000
Average residue weight[3]	112.4
Approximate number of amino acids per mole[3]	1525
Approximate number of peptide bonds per mole[3]	1500
Approximate number of sub-units (polypeptide chains) per mole[4]	25
Approximate average size of sub-units	6800
Approximate average number of amino acids per sub-unit	60
Total number of basic groups by analysis, per mole[5]	177
Ratio of basic amino acids to total number of amino acids	1:10
Total number of hydroxy-amino acids per mole	370
Ratio of hydroxy-amino acids to total number of amino acids	1:4
Total number of sulfur atoms in S-S linkages, per mole	34
Ratio of sulfur atoms in S-S linkages to total number of amino acids	1:45

Constituent	Per cent	Number of residues or atoms per mole
Total nitrogen	16.03	1957
Free α-amino nitrogen	0.21	25
Amide NH_3	1.35	136
Arginine	4.80	46
Histidine	2.50	28
Lysine	6.7	78
Total protein sulfur	1.02	55
Cysteine	0.70	10
Half-cystine	2.37	34
Methionine	1.06	12
Tryptophane	2.86	24
Tyrosine	6.75	64
Serine	11.4	186
Threonine	8.36	120
Leucine	9.32	122

[1] This preparation contained not more than 0.5 per cent of a protein impurity with an electrophoretic mobility different from that of the major component.

[2] A minimum molecular weight of 171,000 can be calculated for this preparation of γ-globulin from the experimental values for the cysteine, half-cystine, methionine, arginine, histidine, threonine, tyrosine, and tryptophane content. The calculations are carried out as described in detail for chymotrypsinogen by Brand, E., and Kassell, B.: Determination of Certain Amino Acids in Chymotrypsinogen, and its Molecular Weight, J. Gen. Physiol., 25, 167–176 (Nov. 1941), and β-lactoglobulin (reference 5 in text). It should be noted that by definition the number of half-cystine residues must always be an even integer (*loc. cit.*).

Svedberg, T. and Pedersen, K. O. (The Ultracentrifuge, Table 48, p. 406, Oxford Press (1940)) have reported a molecular weight of 176,000 for human serum γ-globulin based on sedimentation and diffusion measurements upon the total γ-globulin fraction separated by electrophoresis.

Fraction II, the analysis of which is reported here, does not represent all of the γ-globulin but only a portion thereof. A certain amount of high molecular weight material is present in Fraction II which is thus not homogeneous with respect to size. However, it contains extremely little material of very high molecular weight. (See Paper II of this series.) The best mean molecular weight for this material may be tentatively taken from the osmotic pressure measurements of Scatchard, Batchelder, and Brown as 156,000. (See Paper I of this series.)

[3] For calculation, cf. Brand, E., and Kassell, B., (reference 5 in text).

[4] Equal to the number of free α-amino nitrogen groups, cf. text.

[5] Basic groups are equal to the sum of the free α-amino groups and of the basic amino acids (arginine, histidine, and lysine).

sulfur bridges and by about 110 basic groups per mole (105, 111, and 117 basic groups, *i.e.*, the sum of the α-amino nitrogen, arginine, histidine, and lysine groups for human, bovine, and horse, respectively). The content of free α-amino nitrogen is uniformly high (about 4 per cent of the total nitrogen) and amounts to about 35 such groups per mole. According to a recent hypothesis on the structure of corpuscular proteins (cf. 5, 2), the free α-amino nitrogen may be interpreted as indicating that these molecules do not consist of a single polypeptide chain (folded or coiled), but that they are formed out of a number of shorter chains or "sub-units." Thus, the molecule of serum albumin would be made up out of about 35 "sub-units," which on the average, consist of about 16 amino acids. The teleologist (cf. 1) may wonder whether it is not one of the inherent functions of serum albumin to make available, all through the body, building stones (sub-units) for the easy synthesis of a great variety of proteins.

γ-GLOBULIN

Data on the composition of human γ-globulin are given in Table II in percentage by weight. The number of residues per mole in this table was calculated on the basis of an assumed molecular weight of 171,000. Comparisons of this figure with those derived from physico-chemical measurements on this and other preparations of human γ-globulin are given in Table II, Footnote 2.

In discussing the amino acid make-up of γ-globulin, we are somewhat handicapped since the inferences must be based upon the analysis of the γ-globulin of only one species, man. As was to be expected from the differences in physico-chemical properties (cf. 1), the composition of γ-globulin differs markedly from that of the albumin. Compared to the serum albumins, human γ-globulin contains fewer basic groups (about ½ as many gram per gram) and fewer disulfide linkages (less than ½). On the other hand, the content of tryptophane and of hydroxy-amino acids is very much higher.

The content of free α-amino nitrogen is 0.21 per cent (about 1 per cent of the total nitrogen, compared to 4 per cent in the case of serum albumins), corresponding to about 25 such groups. In terms of the above discussed sub-units hypothesis, this suggests that the molecule of human γ-globulin may be made up out of about 25 sub-units, which on the average would be large (molecular weight about 7,000) and would on the average contain about 60 amino acid residues.

The high content of the hydroxy-amino acids (tyrosine, serine, and threonine) is especially noteworthy; they account for about 22.5 per cent of the weight of this protein. Accordingly, about 1 out of 4 of the constituent amino acids of γ-globulin is a hydroxy-amino acid. It may not be without significance that a similar high content of hydroxy-amino acids is found among the corpuscular proteins only in the 2 protein enzymes, pepsin and chymotrypsin (12). The high content of hydroxy-amino acids is also of interest in connection with Pauling's hypothesis on the rôle of γ-globulin in antibody formation (21).

β-GLOBULIN

A few preliminary data, recently obtained on a fraction rich in β-globulin, are shown in Table III. This sample, isolated in the course of experiments designed to concentrate prothrombin, was reported to contain 11 per cent of α-globulin, 53 per cent of β_1-globulin, 9 per cent of β_2-globulin, and 27 per cent of γ-globulin and was accordingly a complex mixture of proteins of which less than two-thirds had the mobility associated with β-globulin. The sample contained 10.5 per cent of ash for which the analytical data have been corrected.

TABLE III

Preliminary analysis of human β-globulin (prothrombin fraction)

Plasma globulin—Fraction III-2, Prep. GL291

Constituent	Per cent	Moles or atoms per gram $\times 10^5$
Total nitrogen	14.84	1060
Arginine	5.64	32.4
Histidine	2.50	16.1
Total protein sulfur	1.05	32.8
Cysteine	0.2	1.7
Half-cystine	2.5	20.8
Methionine	1.54	10.3
Tryptophane	2.06	10.1
Tyrosine	5.60	30.9
Serine	8.4	80.0
Threonine	7.26	61.0
Leucine	8.9	67.9

Little emphasis can be placed upon the present analytical results, but in general the composition resembles γ-globulin more closely than it does albumin. The hydroxy-amino acids in particular are high and account for about one-fifth of the total.

A more extended analytical study will be carried out when fractions richer in β-globulin become available.

FIBRINOGEN AND FIBRIN

Data on the composition of human fibrinogen and fibrin are given in Table IV, both in percentage by weight and in terms of moles per gram.

Since the fibrinogen preparation analyzed contained about 50 per cent of salt, the results were calculated on the basis of its nitrogen content on the assumption that the nitrogen of fibrinogen was the same as that of fibrin (16.9 per cent). In spite of this analytical difficulty, it can be seen from Table IV that for a number of amino acids, identical results were obtained. The apparent difference in the sulfur and cysteine-cystine content is of questionable significance.

The fundamental question, *i.e.*, whether the nitrogen content and the amino acid composition of fibrinogen and fibrin are identical, remains

unsolved. Conclusive evidence on this point is not as yet available, but it is prerequisite to further advance in the understanding of the chemical reactions of blood clotting.

TABLE IV

Composition of human fibrinogen,[1] human fibrin,[2] and bovine fibrin[3]

Constituent	Human fibrinogen		Human fibrin		Bovine fibrin	
	Per cent	Moles or atoms per gram ×10⁵	Per cent	Moles or atoms per gram ×10⁵	Per cent	Moles or atoms per gram ×10⁵
Total nitrogen	(16.9)[1]		16.9		(17.0)[3]	
Arginine	7.9	45.4	7.9	45.4	8.1	46.5
Histidine	2.8	18.0	2.8	18.0	2.6	16.8
Total protein sulfur	1.26	39.3	1.23	38.4	1.26	39.3
Cysteine	0.41	3.4	0.60	5.0	0.52	4.3
Half-cystine	2.32	19.3	1.81	15.1	2.02	16.8
Methionine	2.52	16.9	2.62	17.6	2.73	18.3
Tryptophane	3.29	16.1	3.22	15.8	3.37	16.5
Tyrosine	5.75	31.8	5.75	31.8	5.70	31.5
Serine	8.3	79.0	9.8	93.3	7.5	71.4
Threonine	6.6	55.4	6.5	54.6	6.8	57.1
Leucine	7.1	54.2	7.1	54.2	7.5	51.2
Glycine[4]					5.70	76.0
Aspartic acid[4]					12.5	93.9
Glutamic acid[4]					14.5	98.6

[1] The preparation of human fibrinogen (No. 81 RI) contained about 47 per cent of protein and 53 per cent of salts; the protein was 87 per cent clottable. The values reported are calculated on the assumption that the content of total nitrogen is the same in fibrinogen as in fibrin (16.9 per cent).

[2] The fibrin (preparation 65A) used for analysis was obtained from a lot of fibrinogen which clotted spontaneously during purification. The clot was extracted with water until free from salts, dehydrated with alcohol, and dried at the laboratory of the Connecticut Agricultural Experiment Station. The nitrogen determination was made by Dr. Jane K. Winternitz of that laboratory.

[3] The bovine fibrin was a commercial preparation. The values given in this table are based on a total nitrogen content of 17.0.

[4] These results were obtained by Rittenberg, D., and Foster, G. L.: A New Procedure for Quantitative Analysis by Isotope Dilution with Application to the Determination of Amino Acids and Fatty Acids, J. Biol. Chem., 133, 737–744 (May 1940), on the same preparation of bovine fibrin. The values reported by these authors have been corrected in Table IV on the basis of a nitrogen content of 17.0 for bovine fibrin.

TABLE V

Amino acid composition of human plasma proteins

	Albumin crystallized from Fraction V	γ-globulin purified from Fraction II	β-globulin concentrate from Fraction III–2[1]	Fibrinogen purified from Fraction I	Fibrin clotted from Fibrinogen[2]
Total nitrogen	15.95	16.03	14.84	16.9	16.9
Free amino nitrogen	0.72	0.21			
Amide nitrogen	1.07	1.35			
Arginine	6.15	4.80	5.64	7.9	7.9
Histidine	3.52	2.50	2.50	2.8	2.8
Lysine	5.8	6.7			
Total protein sulfur	1.96	1.02	1.05	1.26	1.23
Cysteine	0.70	0.70	0.20	0.41	0.60
Half-cystine	5.58	2.37	2.50	2.32	1.81
Methionine	1.28	1.06	1.54	2.52	2.62
Tryptophane	0.19	2.86	2.06	3.29	3.22
Tyrosine	4.66	6.75	5.60	5.75	5.75
Serine	3.7	11.4	8.4	8.3	9.8
Threonine	5.03	8.36	7.26	6.6	6.5
Hydroxyproline	0				
Leucine	11.9	9.32	8.9	7.1	7.1

[1] The β-globulin, unlike the albumin and γ-globulin preparations, is a concentrate and not a purified protein. It contained 62 per cent of β-globulin, 11 per cent of α-globulin, and 27 per cent of γ-globulin. It was extracted with ether to remove lipid components. The values are included in this report to permit preliminary comparison with the albumin and γ-globulin.

[2] The fibrin preparation was purified by extracting the clot extensively with water and alcohol. It contained 0.2 per cent of ash.

In order to permit ready comparison of the composition of the human plasma proteins, one with the other, the amino acid composition expressed in percentage has been set out in Table V. These data will be revised and amplified and extended to other plasma proteins as opportunity offers.

The data presented in this paper, incomplete as they are, have yielded some interesting implications, which should prove to be of practical use. The necessity for more detailed information on the chemical composition of all of the various plasma fractions is clearly indicated.

SUMMARY

As a preliminary contribution to the understanding of the composition of the human plasma

proteins, amino acid analyses have been carried out on normal human serum albumin, the human γ-globulins of importance in immunity, a fraction rich in β-globulin, and human fibrinogen and fibrin. Comparable analytical studies on the serum albumin of cattle and the horse are included for comparison. Highly significant differences in the amino acid distribution have been noted, among them being the high percentage of hydroxy amino acids in the γ-globulins, the very low content of tryptophane in the albumins, and the absence of methionine in horse serum albumin. Knowledge of the amino acid composition of the plasma proteins is essential from the point of view of nutrition, as well as for the understanding of the regeneration of plasma proteins. It is also of fundamental importance to a knowledge of the reactions that take place between proteins and other substances.

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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

IV. A STUDY OF THE THERMAL STABILITY OF HUMAN SERUM ALBUMIN[1,2,3]

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Normal human serum albumin, though prepared as a white dry powder, is distributed to the armed forces as a 25 per cent aqueous solution. This is advantageous, since the solution is ready without reconstitution for immediate emergency use, and is possible, because the albumin solution is very stable, and, for a protein, very resistant to heat.

A 25 per cent solution of human serum albumin is a clear, straw-colored liquid. Its viscosity is about the same as that of whole blood with a hematocrit of 50. On standing at very high temperatures, it in time becomes more viscous, and haze with or without visible flocs appears. Later still, gelation occurs. The rate of these changes increases with increase in temperature. The intravenous kit includes a 200 mesh filter to remove flocs, and there is no evidence that the usefulness of the solution is impaired as long as it will flow through the needle. However, haze might be interpreted by the medical officer in the field as bacterial contamination and the development of flocs or of increased viscosity indicates incipient denaturation of protein. Excessively rapid denaturation probably indicates some impurity other than albumin in the original preparation. Therefore, the initial turbidity and its rate of change at 50° C. (122° F.) and 57° C. (135° F.) have been made the criteria of the acceptability of human serum albumin solutions for the armed forces. In addition, we have used the change in viscosity as a measure of the stability of albumin solutions. The two are complementary since turbidity measures the size of individual particles and depends but little on their shape or degree of hydration, while viscosity measures the shape and degree of hydration and depends but little on the size. The stability of albumin is so great that it has been necessary to devise tests which subject the albumin to much more rigorous conditions than it is likely to meet in the field, and measure changes much too small of themselves to be of practical importance.

METHODS

Viscometric studies have been made in an Ostwald type viscometer, modified so that sterility may be maintained and evaporation from the warm surfaces prevented. In this method, the density and time of flow through a capillary are measured. The viscosity is proportional to the product of density and time, and the proportionality constant can be determined by measuring these quantities for a liquid of known viscosity. In a stability study, the density remains constant, so viscosity is proportional to time of flow.

In dilute solutions, the viscosity, η, and its logarithm are both proportional to concentration, but the logarithm remains proportional up to much higher concentrations, and is thus a better measure of the extent of such change. Those reported in this paper are so small that this difference is not important, but we have followed our usual custom and tabulated values of $\log \eta$.

Turbidity studies have usually been made in a Zeiss-

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[1] This work has been carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[2] This paper is Number 15 in the series "Studies on Plasma Proteins" from the Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross.

[3] This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the United States Navy. The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting views of the Navy Department or the Naval Service at large.

[4] Lieutenant Commander, Medical Corps, United States Naval Reserve.

Pulfrich photometer with nephelometer attachment, in which the scattered beam makes an angle of 45° with the unscattered emergent beam.[5] A very slight visible haze corresponds to 20 or 25 units, and an easily visible haze to 30 or more units. The average well-filtered albumin solution has a nephelometric reading of less than 10 units, even after being heated at 50° C. for 2 weeks. An increment of 20 nephelometric units would thus render the average preparation hazy and has been adopted as a convenient quantitative measure of stability. Some of the solutions have been studied in a special photoelectric tyndallometer [6] which measures the light scattered at any angle. The light source is a high pressure mercury arc. The incident beam can be polarized at any angle and the intensity of its polarized components measured. One unit of this instrument is approximately one-fifth of a Zeiss unit.

In dilute solutions, the scattered light is proportional to the concentration of scattering particles, but at higher concentrations, the effect of the solute on the index of refraction becomes important. In concentrated albumin solutions, the scattering actually decreases as the protein concentration increases. However, there is little change of index of refraction on denaturation, and the increase in turbidity is proportional to the number of scattering particles if the nature of these particles is unchanged.

The light scattering per unit mass depends greatly upon the size of the particles, is at a maximum for particles whose diameter is equal to the wave-length of light (about 5000 Å), and is very small for diameters less than one-tenth or more than ten times this wave-length. For particles smaller than the wave-length of light, the scattering at 45° is approximately twice that at 90°, but larger particles scatter much more through the smaller angle. So the contribution to the scattering of large particles may be determined approximately from the difference in scattering at these two angles. It may be recalled in this connection that the serum albumin molecule is approximately egg-shaped and is estimated to be about 150 Å in length and 38 Å wide (Studies I, Table I) (2).

J. Murray Luck and his associates at Stanford University have devised a turbidity test for the stability at high temperatures by measuring the time necessary to give a visible cloud in a capillary tube illuminated from the side. The turbidity increase at this stage is so rapid that a very approximate measure of turbidity gives an accurate measure of the time. This method is discussed in another paper in this series (1).

Gelation studies have also been undertaken to determine, in quantitative terms, the relation between turbidity and development of structure. This has been estimated by noting the time at which freedom of motion of entrapped air bubbles is lost. The end-point adopted is that the bubbles in a bottle containing 25 per cent albumin, when rotated back and forth, shall exhibit recoil. Whereas gelation limits the conditions to which these very stable albumin solutions can be subjected, incipient turbidity gives the earliest indication of instability, and has indeed formed the basis for the testing and acceptance of albumin for the armed forces.

The effects on stability at different temperatures, of changing the concentrations of albumin, of salt, of hydrogen ion, and of merthiolate, and of storing at moderate temperatures have been determined. Most of these effects were studied first on commercial preparations in which 1 to 2 per cent of the protein was globulin, but many of our more detailed experiments have been carried out on crystallized human albumin of very high purity.[7] Such studies tell us more regarding the albumin itself. The crystallized albumin solutions studied are identified as HA 42, HA 64, and COM 1. The standard albumin preparations from different laboratories are identified by capital letters.

RESULTS

Effect of pH on stability

Early experiments with standard albumin, prepared by the method of Cohn and coworkers (2), showed that there is a maximum stability at approximately 6.8. More careful experiments have now been carried out on 2 samples of crystallized albumin.[8] The stabilities, defined as the time necessary for an increase of 20 units in the nephelometer reading (20 N.U.) and for an increase of 10 per cent in viscosity ($\Delta \log \eta = 0.0414$), are given in Table I and graphically represented in Figure 1, which gives the relation of stability

[5] The instrument is used with a green filter whose maximum transmission is at 5300 Å. Each nephelometric unit is approximately 1 per cent of the turbidity of a standard whose absolute turbidity is given as 0.0193. By removing the filter and focusing on the solution, this instrument can also be used to study visible flocs under standard conditions.

[6] This tyndallometer, designed by Professor Hans Mueller and Dr. George Rado, has been modified by them by omission of the polarization features and limitation to one of three angles, 0°, 45°, and 90° of the scattered light, to yield a simplified instrument for use in the study and acceptance of normal human serum albumin, under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Massachusetts Institute of Technology.

[7] These preparations were crystallized by the method of Cohn and Hughes at the Harvard pilot plant.

[8] A concentrated solution of crystalline albumin, HA 42, was prepared and filtered through a Seitz Ser. 3 pad. From this stock solution, other sterile solutions were prepared with different concentrations of protein, salt, and hydrogen ion. The hydrogen ion concentration was varied by adding sodium bicarbonate and then reducing the carbon dioxide concentration approximately to that in equilibrium with air by equilibrating in a tonometer for an hour.

of HA 42 to pH for the solutions of 25 per cent albumin and 0.2 M sodium chloride.

An increase of 20 nephelometric units, although a somewhat arbitrary choice, is a measure of the reading of a solution having a barely visible haze. The choice of 5 per cent or 10 per cent for the increase of viscosity is entirely arbitrary. However, the shapes of the curves are usually so similar that doubling or halving the arbitrary values would make little change in the relative stabilities (Figure 2).

The curves in Figure 1 represent the experimental points satisfactorily except that the point at pH 6.60 appears to show too great nephelometric stability at 57° and too little at 50°. The curves of nephelometric reading versus time for both of these measurements were not of regular form, as shown in Figure 2, so that these points are probably not as certain as the others. Each curve in Figure 1 is symmetrical and of the same form. Each decreases 4 per cent of the maximum value at 0.2 pH unit either side of the maximum and 25 per cent at 0.5 unit from the maximum. The maximum is at 6.75 for viscosity at 55°, 6.92 for nephelometry at 57°, and 7.02 for nephelometry at 50°.

In these experiments, the pH adjustment and CO_2 equilibration followed filtration to avoid variations from filtration. Another set of solutions was therefore prepared from crystalline albumin, HA 64, and each was filtered after equilibration. They were studied nephelometrically at 50° and 57° and viscometrically at 55° and 57°. The composition, pH, and stability of the solutions used are given in Table II. Figure 2 shows curves of nephelometer readings and changes in the logarithm of the viscosity, $\Delta \log \eta$, at 57°. The maxima at 57° are at pH 6.75 for nephelometry and at 6.70 for viscometry. At the lower temperatures, the nephelometric maximum appears to be at a slightly higher pH and the viscometric maximum at a slightly lower one. The maxima are not quite as flat as those for HA 42, but the stability is again greater than 90 per cent of the maximum, *within 0.2 pH* units of the maximum.

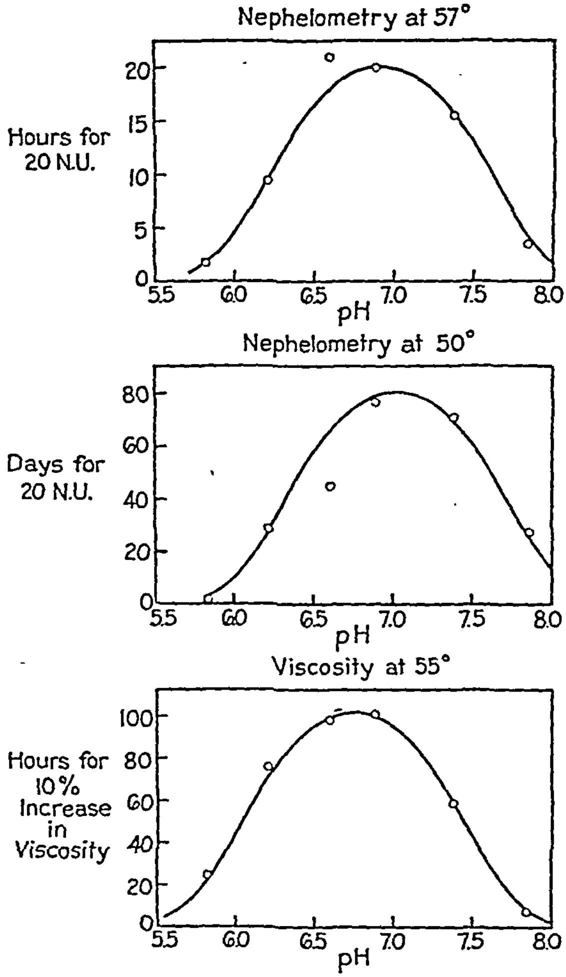

FIG. 1. STABILITY OF CRYSTALLIZED HUMAN ALBUMIN (HA 42)

TABLE I

Stability of crystallized human albumin (HA 42)

pH	Albumin	NaCl	For increment of 10 per cent in viscosity at 54.7°	For increment of 20 N.U. at 57°	For increment of 20 N.U. at 50°
	per cent	*moles per L.*	*hours*	*hours*	*days*
5.83	25.04	0.2045	24	1.7	1.6
6.21	24.72	0.2012	75	9.5	28.5
6.60	24.95	0.2156	98	21.0	45
6.69	24.91	0.1876	98	18.5	68
6.76	27.06	0.2040	96*	16.5*	16*
6.77	20.04	0.1897	110*	27.5*	2.5*
6.79	24.83	0.3075	144	30.0	142
6.88	25.02	0.2020	101	20.0	76.5
7.37	25.27	0.2015	59	15.5	70.5
7.84	25.05	0.2131	7.5	3.5	27.5

* Time for change equivalent to 20 N.U. or 10 per cent in viscosity for 25 per cent albumin.

Effect of salt concentration on stability

Early studies on standard commercial albumin showed that the nephelometric stability is

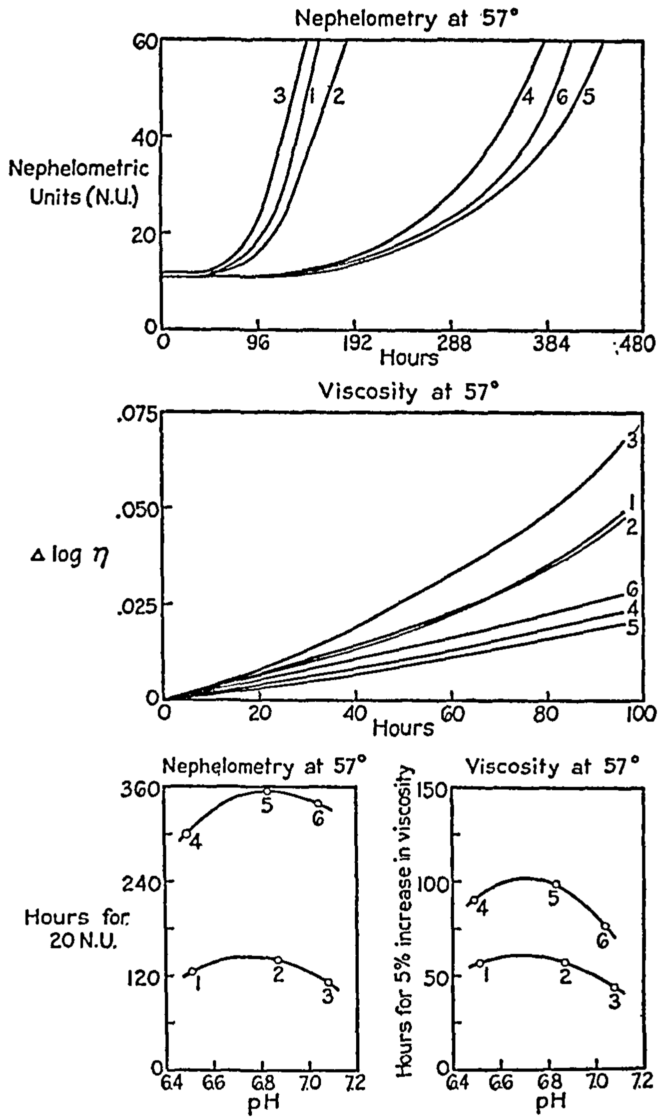

FIG. 2. STABILITY OF CRYSTALLIZED HUMAN ALBUMIN (HA 64)

proportional to the salt concentration from 0.15 M to 0.6 M NaCl, but these results alone could not determine if there was a real decrease in denaturation or if the increased salt merely held in solution the denatured protein. Experiments on HA 42 and HA 64, represented in Tables I and II and Figure 2, indicate approximately the same relationship, with perhaps a larger effect on the nephelometric stability than on the viscometric. The combination of viscometry and nephelometry shows that increase of salt concentration does give an important decrease in denaturation.

J. Murray Luck and his colleagues at Stanford University have undertaken the study of the effect of salts other than sodium chloride on the stability of albumin. Their results appear in another place (1).

Effect of albumin concentration on stability

The effect of albumin concentration on stability is illustrated in the last 2 experiments of Table I. To study this effect further, a 25 per cent solution of HA 42 with 0.2 M NaCl at pH 6.77 was diluted to $\frac{3}{4}$, $\frac{1}{2}$, and $\frac{1}{4}$, and the viscosity of each solution was determined. The solutions were heated together at 57° for 48 hours, and the viscosities redetermined. The most concentrated solution was diluted to the concentrations of the other solutions, and the viscosities of these solutions were also determined.

The results are summarized in Table III, expressed as $1/C \log \eta/\eta_0$. For the unheated solutions, this quantity decreases slightly with decreasing concentration. The change in $1/C \times \log \eta/\eta_0$ on heating at 25 per cent and diluting is almost independent of the concentration, but the change on diluting and heating at C per cent decreases very rapidly. It is, in fact, nearly proportional to the cube of the concentration. So the change in viscosity is much greater for the first process. It is quite possible that the initial denaturation changes quite differently with concentration and that we are measuring merely the chances of a molecule in a state of nascent denaturation colliding in the proper way with another molecule before it adjusts the change intermolecularly.

These stability considerations, presented in

TABLE II

Stability of crystallized human albumin (HA 64)

Preparation	pH	Albumin	NaCl	Time for increment of 5 per cent in viscosity		Time for increment of 20 nephelometric units	
				at 57.2°	at 54.7°	at 57°	at 50°
		per cent	*moles per L.*	*hours*	*hours*	*hours*	*days*
1	6.51	26.88	0.148	58	129	125	116
2	6.87	26.59	0.148	56	101	140	176
3	7.08	26.44	0.149	44	87.5	113	167
4	6.49	26.67	0.293	90	160	300	226
5	6.83	26.58	0.292	100	145	355	(136)*
6	7.04	26.45	0.289	76	112	340	262

* Measurement probably in error, see text.

(31) showed "that rabbit thrombin is of distinct value in the control of hemorrhage from *small wounds*." Similar preparations have now been obtained from the plasma of man and of several other animals in addition to the rabbit (32).

The human thrombin preparations obtained in these laboratories, by the conversion of the prothrombin in Fraction III-2 with human thromboplastin of placental origin, consist predominantly of β-globulin, to the extent of about 75 per cent as determined by electrophoresis. It is not yet possible to state, however, that thrombin is itself a β-globulin, since thrombin certainly makes up only a small part of the total protein present in the preparation. Solutions of this material retain their activity for several days in the cold. Thrombin solution may be filtered into a sterile container, frozen, and dried. The resulting white powder remains stable over a protracted period of time [9] when preserved in the dry state and redissolves readily on the addition of saline solution, forming a slightly opalescent liquid of high thrombic activity. The activity is of the order of 15 to 20 units of thrombin per milligram of protein in the dry powder. Whereas this preparation has thus far less activity per milligram [10] than the highly purified preparations of Seegers and Milstone, its potency lies far above minimal requirements for clinical use, and the impurities present in our preparation, as well as the thrombin itself, are all proteins of human origin, so that there is no obvious practical advantage to be gained by further purification for clinical use.[11]

THROMBOPLASTIN

The conversion of prothrombin to thrombin is ordinarily very slow and incomplete except in the presence of calcium ions and of an activating material of the class commonly termed thromboplastin (or thrombokinase).[12,13] It now appears clear that certain thromboplastins, at least, are lipoproteins. The one most thoroughly studied is that obtained from the lungs of cattle and investigated by Chargaff and his collaborators (33 to 36). The active protein material is readily precipitated at low salt concentrations in the neighborhood of pH 5.1. The lipids could be extracted from the protein with a mixture of boiling alcohol and ether; the lipid-free protein, after this extraction, showed no thromboplastic activity. The lipid components were shown to contain a number of fatty acids, both saturated and unsaturated; also a large amount of choline and a small amount of ethanolamine, together with other unidentified bases (possibly serine). The union between the lipid and the protein in the material freshly obtained from lung appears to be very firm. Recent electrophoretic studies (33 to 36) have shown that the thromboplastin in solution migrates with a single boundary in the Tiselius apparatus at pH 7.4 and pH 8.8. A small fraction of the preparation, which contains nucleic acid, moves with a different velocity and can thus be separated by electrophoresis. The nucleic acid fraction is apparently not necessary for the thromboplastic activity.

The thromboplastic protein is most effectively separated by sedimentation in a preparative ultracentrifuge, employing centrifugal accelerations about 30,000 times gravity (33 to 36); the precipitate so obtained redissolves readily in a borate buffer at pH 8.6. Its sedimentation constant is reported (35) to be near 330 Svedberg

[9] Recent tests on one preparation show no loss of thrombic activity on heating for 115 days at 50° C. Further thermal stability tests on this and other preparations are being carried out.

[10] As explained in footnote 8, the units employed by us may not be strictly comparable with those of other authors. We are inclined to believe that our unit is larger than that of Seegers, but a precise comparison of the two has not yet been carried out.

[11] The thromboplastin employed (see the discussion below) in converting prothrombin to thrombin is obtained from human placentas, thus no protein of non-human origin is involved in the preparation.

[12] Milstone (28) has recently made the striking observation that concentrated prothrombin solutions are slowly converted to thrombin in 0.1 saturated ammonium sulfate at pH 5.4. The solution, after dialysis against ammonium sulfate solution in the cold, was kept one week at 20°, when conversion was complete, and then stored at 1°. No added activator was required, and the addition of oxalate did not retard the process of conversion. As yet, we have not found it possible to employ a "spontaneous" conversion of this type in the preparation of human thrombin.

[13] It is impossible in this place to give any adequate discussion of the extensive literature concerning thromboplastin. The reviews cited (1 to 6), especially Chapter V of the monograph by Quick (5), supply extensive references on the subject.

units, and its molecular weight to be close to 170 million; it contains approximately 8 per cent nitrogen and 1.4 per cent phosphorus. The thromboplastic activity is so great that as little as 0.008 μgm. markedly accelerates the clotting of 0.1 cc. of rooster plasma; the preparations also show marked phosphatase activity. In its large size and high lipid content, this material appears similar to the "microsomes" obtained by Claude (37, 38) from the cytoplasm of many tissues, although as yet no thromboplastic activity has been reported for the latter.

The thromboplastic lipoprotein of lung can be made to dissociate by the action of heparin (33 to 36), but in that this report is not intended to treat the very numerous communications dealing with the function of heparin in prevention of coagulation, the possible implications of this finding lie outside its province.

A tissue globulin having high thromboplastic activity has been obtained from human placentas (39); it is insoluble at pH 5.2, and soluble at pH values alkaline to 7. It appears, however, to be a very large molecule, and is removed by passing the material through a Sharples centrifuge. Thromboplastic activity is rapidly lost in acid or alkaline solutions, and more slowly by oxidation or by aging, especially in the presence of fresh serum. The properties of this material are similar to those of the thromboplastic protein from lung; but chemical analyses of the placental protein are not yet available. It is reported to be of value in the treatment of hemophilia (39).

This placental thromboplastin has been used in our laboratory to convert human prothrombin to thrombin in the presence of calcium ions. It has proved to be a highly efficient converting agent, and is stable for a period of at least several weeks if kept at 0 degrees with a small amount of tricresol as a preservative. Although its activity is not quite so high as that of some thromboplastins of animal origin, it has the great advantage of containing only human material, and therefore introduces no heterologous protein when added to human plasma protein fractions.[14]

[14] We are indebted to Miss K. Fahey of the Children's Hospital, Boston, for preparing this material and supplying it to us.

THE "GLOBULIN SUBSTANCE" WHICH PROMOTES THE CLOTTING OF HEMOPHILIC BLOOD

In 1937, Patek and Taylor (40) obtained from normal human plasma, by dilution and acidification, a globulin fraction which greatly accelerated the clotting of hemophilic blood. The most active fractions were those precipitated at pH 5.5 to 6.0. Similar fractions prepared from hemophilic blood were inactive or very weakly active. The active factor was thermolabile, and soluble in isotonic saline. An active preparation could also be obtained by the removal of salts by dialysis from normal human plasma (41). The euglobulin so obtained was active in reducing the coagulation time of hemophilic blood, both *in vitro* and *in vivo*. The active factor was not identical with prothrombin or thrombin. It also appears different from tissue thromboplastins, since it passes readily through a Berkefeld filter and does not shorten the clotting time of normal blood.

We are indebted to Dr. F. H. L. Taylor and his associates at the Thorndike Memorial Laboratory of the Boston City Hospital for testing the plasma fractions separated at the Harvard pilot plant for the presence of this clotting factor. It was found that the active factor was present to a very large extent in Fraction I. The rest was almost all concentrated in Fraction II + III, and could be further subfractionated in Fraction III-2. Prothrombin, as we have seen, is also concentrated in this fraction; but the reported data (40, 41) for the fractions which shorten the clotting time of hemophilic blood suggest that the active component is not so labile as prothrombin.

FIBRINOLYSIN

That a slow dissolution of the fibrin clot occurs, even under completely sterile conditions, has long been known (42). The time required for this dissolution, however, varies greatly in different clots; it may vary from a few hours, or even less, to 2 or 3 weeks. A typical clot obtained from Fraction I, formed under sterile conditions in the presence of a minute amount of thrombin, dissolves in the course of 1 to 10 days, the time required for dissolution depending on pH, temperature, and other factors. A sensitive

optical method for following the course of fibrinolysis by relative turbidimetry, using the Evelyn photoelectric colorimeter, has recently been described by Ferguson (43, 44).

The speed of fibrinolysis is greatly increased if large amounts of Fraction III-2, or of the thrombin prepared from this fraction, are added to the clot. Thus, the addition of large amounts of the human plasma fraction containing thrombin accelerates both the formation of the clot and its subsequent dissolution. When very large amounts of Fraction III-2 are added, relative to the amount of fibrin in the system, lysis may begin within a few minutes after the clot is formed, and be complete within half an hour. There is good reason to believe, however, that the lytic factor is not identical with prothrombin or thrombin.[15]

The fibrinolytic power is remarkably enhanced if plasma or certain plasma fractions are well shaken with chloroform, and the mixture allowed to stand for a period of from one hour to several days. On removal of the chloroform, the resulting serum is found to dissolve fibrin clots with extraordinary rapidity. The earlier work in this field has recently been summarized by Tagnon (45), who has also shown that lysis of fibrinogen, as well as of fibrin occurs, so that chloroform serum, even when it contains small amounts of thrombin, may not cause clotting of fibrinogen but simply a breakdown of the fibrinogen molecule which renders the solution incapable of clotting when more thrombin is added. The activity of the activated enzyme is inhibited by plasma fractions containing high concentrations of prothrombin, but there is as yet no proof that prothrombin is itself an inhibitor. The action of the enzyme is definitely proteolytic, since it is capable of digesting fibrinogen, fibrin, gelatin, and casein, as indicated by the progressive formation of non-protein nitrogen when it acts upon these substrates (46, 47). It also causes a progressive decrease in the viscosity of gelatin. Its optimum activity is at pH 7.4, and it is inactivated by heat.[16]

Tagnon (45, 46) has emphasized the fact that considerable amounts of thrombin may be found in chloroform treated plasma, previously freed of calcium and platelets, or other sources of thromboplastin. He believes that the enzyme itself may function as the active agent for converting prothrombin to thrombin, although the thrombin so formed may be subsequently destroyed on prolonged standing, through the proteolytic activity of the enzyme. In recent years, Ferguson (48) has independently been developing a similar view, which assigns to a proteolytic enzyme of plasma—termed by Ferguson serum-tryptase—a key position in the initiation of the clotting process. In this connection, the demonstration by Eagle and Harris[17] that crystalline trypsin converts prothrombin to thrombin is certainly highly suggestive.

It is impossible to give at present any final evaluation of these conceptions. If the enzyme functions as the essential agent for converting prothrombin to thrombin, then it must be released from an inactive form by some natural activator which performs the same function which is subserved by chloroform *in vitro*. While the blood remains fluid as it circulates in the body, the action of this activator must be held in abeyance. Thus, a still unknown mechanism must be postulated, and its nature explored, if the proteolytic enzyme theory of clotting is to be accepted. In this communication, however, we are not directly concerned with the theory of clotting, but rather with the preparation of active and stable materials, which act on different phases of the blood clotting mechanism, and which are susceptible of possible clinical use.

SUMMARY

In the course of the large scale fractionation of human plasma, preparations containing certain of the proteins concerned in the blood co-

[15] Evidence bearing on this point is given by Wöhlisch (2), pp. 363–368.

[16] Determinations on our human plasma protein fractions by Dr. Taylor and his associates, together with measurements from this laboratory, indicate that the enzyme precursor which can be activated by chloroform is found in several different plasma protein fractions—some of it in Fraction I, and larger amounts in Fractions II + III and IV. On subfractionation of II + III, most of it is concentrated in Fraction III-2; on subfractionation of IV, most of it goes into Fraction IV-1. The distribution of the enzyme among different fractions appears to be somewhat variable from one preparation to another.

[17] See Eagle (1), p. 106.

agulation mechanism have been obtained. Outstanding among these are (*1*) fibrinogen, which is concentrated in Fraction I, which can be further purified when this is desired for special purposes, (*2*) thrombin, prepared from the prothrombin which is concentrated in Fraction III-2, by conversion with human placental thromboplastin. Fraction I, purified fibrinogen, and thrombin have all been prepared in dry, stable and sterile form; and they serve as starting materials for the making of a series of products, the properties and uses of which are described in subsequent papers of this series. The properties of certain other substances, related to the clotting of blood and the dissolution of blood clots, are also discussed; and the chemical properties and functions of all these substances are briefly considered.

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Table III, would favor reducing the albumin concentration. The importance of the space and weight saved by using concentrated solutions is so great however, that there can be no reduction of the concentration for transport for the armed forces as long as sufficient stability can be attained by other means. There will sometimes be a great advantage in a highly hypertonic solution but sometimes an isotonic solution will be preferred. The stability will have but little influence on the choice of albumin concentration.

Effect of merthiolate concentration on stability

Various mercury-containing chemicals, such as merthiolate or phenyl mercuric borate, are used as preservatives in standard commercial preparations of serum albumin. The preparations of crystallized albumin which we have studied contained no preservative. To determine whether this difference might cause differences in stability, solutions of crystallized albumin, COM 1, were prepared, at a pH of 6.75, containing 25 per cent albumin, 0.15 M NaCl, and 0, 1:10,000, or 1:5,000 merthiolate. The viscometric stabilities at 55° C. were 93, 98, and 97 hours. Our results indicate that merthiolate does not appreciably affect stability, and that the choice of the concentration of merthiolate, and probably of other mercurials, need not be influenced by stability considerations.

Effect of storage at moderate temperatures on stability

Preparations of albumin which have been stored at 0 degrees show no change in stability, either at that temperature or when exposed to high temperature. Those which have been kept at room temperature show no change in viscosity or nephelometry even after very long times. When later exposed to high temperatures, however, they show a somewhat decreased stability. Experiments on a standard commercial preparation and on a crystallized preparation stored at 0°, 25°, and 37° confirm these results. We will give details only of the latter.

TABLE III

Stability of crystallized human albumin (HA 42 at 57°)

Albumin	$\frac{1}{C}\log\frac{\eta}{\eta_0}$ unheated	$\frac{1}{C}\Delta\log\frac{\eta}{\eta_0}$ heated at 25 per cent	$\frac{1}{C}\Delta\log\frac{\eta}{\eta_0}$ heated at C per cent
per cent			
25.0	0.0309	0.0048	0.0048
19.0	0.0265	0.0048	0.0022
14.0	0 0237	0.0047	0.0012
9.0	0.0214	0.0046	0.0001

TABLE IV

Stability of crystallized human albumin (HA 64)

Stability at 0°, 25°, 37° C.
Ratio of viscosity at 100, 200, or 400 days to initial viscosity

NaCl pH		0.15 6.8	0.15 7.0	0.30 6.8	0.30 7.0
days	° C.				
0		1.00	1.00	1.00	1.00
100	0	0.99	1.01	1.01	0.99
200	0	0.99	1.00	1.01	1.00
400	0	0.99	1.00	1.01	1.00
100	25	0.99	1.01	1.01	0.99
200	25	1.00	1.01	1.01	1.00
400	25	1.01	1.02	1.01	1.00
100	37	1.00	1.02	1.03	1.01
200	37	1.02	1.03	1.03	1.02
400	37	1.06	1.04	1.06	1.04

Solutions of HA 64, at pH 6.8 and 7.0 and NaCl concentrations 0.15 M and 0.3 M, were stored in several small bottles at 0°, 25°, and 37° C. At a specified time, a bottle of each was withdrawn, and its stability at 57° determined viscometrically and nephelometrically.[9] The results for 400 days are given in Tables IV and V.

Table IV shows the ratio of the viscosity after storage to that before storage. At 0° and 25°, there is no increase even in 400 days. At 37°, there appears to be a small increase which is, however, greater than the experimental error.

Table V shows the effect on the 57° stability of storage at lower temperatures. At 0°, there is no marked difference. (The nephelometric value at zero time for pH 6.8 and 0.3 M NaCl may be regarded as abnormally high.) At 25° and 37°, there is a marked decrease in the 57° stability. The decrease is larger at 37° than at 25°, larger for 0.15 M NaCl than for 0.3 M, and somewhat larger for pH 6.8 than for pH 7.0.

[9] In order to save material, the light scattering was measured by Dr. George Rado in the tyndallometer of Dr. Hans Mueller.

TABLE V

Stability of crystallized human albumin (HA 64)
Effect of storage at 0°, 25°, and 37° C. on stability at 57° C.

NaCl pH		0.15 6.8	0.15 7.0	0.30 6.8	0.30 7.0	0.15 6.8	0.15 7.0	0.30 6.8	0.30 7.0
		For increase of 50 Mueller units				For 5 per cent increase in viscosity			
days	*° C.*	*days*				*half-days*			
0		4	3	10	6	4	4	9	7
100	0	4	3	8	7	5	3	10	7
200	0	4	3	8	8	5	4	10	8
400	0					5	3	10	7
100	25	3	2	6	5	3	2	8	6
200	25	2	2	4	5	2	2	6	5
400	25	2	2			2	2	5	6
100	37	3	2	5	5	3	2	7	7
200	37	1	1	3	4	2	2	5	5
400	37	0.5	0.5			0.5	0.5	2	3

These results, as well as those graphically represented in Figure 2, show a marked similarity between the nephelometric reading and the viscosity, in that both begin to change rapidly at the same time. They also show an important difference in that the nephelometric reading remains constant before this rapid change while the viscosity changes from the very start. The relative stabilities could be determined as well from the initial slopes of the viscosity curves as from the later values. This indicates that there is a change in shape before there is any formation of molecules large enough to give much light scattering.

The experiments on storage at moderate temperatures and on filtration, considered in subsequent sections of this paper, indicate that the very first effect is one which affects neither light scattering nor viscosity but only renders the material more susceptible to changes which register by our methods of study. Our results suggest that this first reaction varies less rapidly with the temperature than the subsequent ones, so that it is not negligible at room temperature, and also that the effect of albumin concentration on stability is exerted on the subsequent stages by decreasing the probability of encounters of susceptible molecules. Many careful experiments would be required to establish these points.

Effect of storage at high temperatures on stability

The thermal stability of every commercial preparation has been tested on samples submitted to the Harvard Albumin Control Laboratory. These samples have been incubated routinely at 50° and, during the past 8 months, at 57° C. as well. Nephelometric readings, as previously described, have been made at frequent intervals, and the rate of increase in turbidity

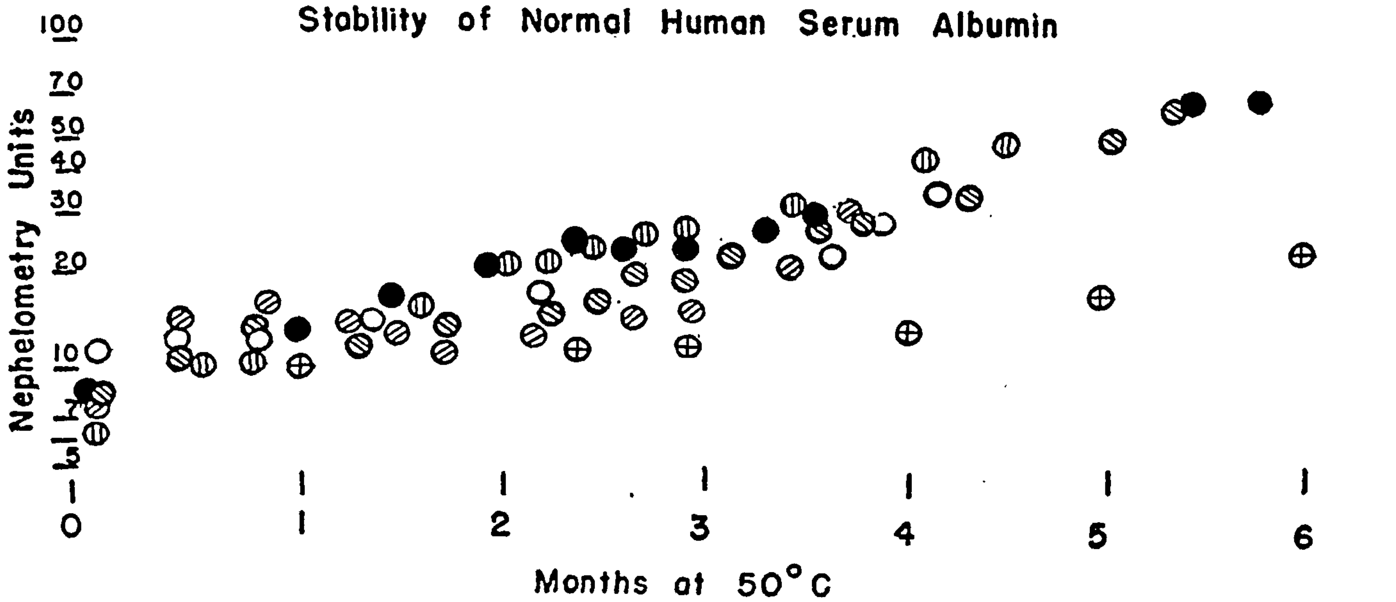

FIG. 3. NEPHELOMETRY RESULTS ON AN AMORPHOUS PREPARATION OF ALBUMIN FROM EACH OF 5 LABORATORIES COMPARED WITH A CRYSTALLIZED PREPARATION (⊕)

determined. Samples both at 50° C. and 57° C. have been kept in the oven until gelation occurred. From these tests, it has been possible to show not only that 25 per cent serum albumin is a remarkably stable protein preparation, but that during the first 2 years of production, there has been a great improvement in the thermal stability of the albumin from all laboratories.

The most marked increases in stability have occurred (*1*) following the adoption of the pH which has been proven optimal for stability, and (*2*) following the adoption of a salt content of 0.3 M (1.7 per cent NaCl) rather than 0.15 M (0.85 per cent NaCl) for the 25 per cent albumin solution. The stability of the standard, commerical, amorphous albumin preparations studied (containing less than 2 per cent globulin) was, however, lower than that obtained with the experimental crystalline lots processed at the Harvard pilot plant. The stability of crystallized lots should represent nearly the maximum attainable under given conditions of salt content, pH, and temperature.

The nephelometric readings from a study of one standard preparation from each commercial laboratory, which has been incubated at 50° C. over a period of months, are graphically represented in Figure 3. Results from the study of crystallized albumin are also charted. Not only the crystallized, but also the standard albumin is quite usable even after 6 months at 50° C. At 57° C., the number of hours necessary to cause an increase of 20 nephelometric units, based on the average of all preparations approved for delivery to the armed forces in the past 6 months, is over 80 hours and for the best preparations over 100 hours.

There is no evidence that the development of the slight haze represented by the highest nephelometric reading recorded in Figure 3 would be injurious clinically. Such preparations have been used without reactions of any kind being noted.

These very careful studies were therefore undertaken to render the preparations (*1*) as nearly uniform as possible, and (*2*) as stable as possible even when exposed to the most adverse conditions likely to be imposed by military medicine.

The relation of turbidity to gelation at high temperatures

Albumin would clearly not be usable in the field if incipient gelation occurred, as flow through the filter and needle would be inhibited. It therefore becomes important to determine the relation between length of time for a standard albumin solution to show haze at a high temperature, *i.e.*, to increase 20 units at 57° and to show incipient gelation. A study of over 150

FIG. 4. THE RELATION OF NEPHELOMETRY TO GELATION IN 150 PREPARATIONS OF NORMAL HUMAN SERUM ALBUMIN

TABLE VI

Effect of filtration on heated albumin

HA 35, pH 6.70	Albumin	NaCl	Albumin	Nephelometric reading	Viscosity	Nephelometric stability at 57°	Viscometric stability at 54.7° C.
	per cent	*moles per liter*	*kgm. per mole NaCl*		*at 25 per cent albumin*	*hours*	*hours*
Unheated	25.3	0.161	1.58	6.5	5.66	10	41.5
Heated 11 hours at 57° unfiltered	25.3	0.161	1.58	36.	6.88		
Heated 13.5 hours at 57° unfiltered				65.	7.03		10.5
Heated 16 hours at 57° unfiltered	25.3	0.161	1.58	78.	7.43		
Heated 11 hours at 57° refiltered	25.6	0.160	1.61	13.5	6.37	3.5	24.5
Heated 16 hours at 57° refiltered	25.5	0.162	1.57	21.	6.88		

standard albumin preparations is reported graphically in Figure 4. There is considerable spread in the results, due in part to variations within the permissible pH range and in part to slight differences in practice in the 7 different laboratories which processed the albumin. Only in the case of unsatisfactory preparations which became hazy in less than 2 days did gelation occur soon after the first visible haze. On the average, the later preparations appear to take about three times as long for incipient gelation as for the formation of the first visible haze.

Filtration of partially denatured albumin

It may become important to know whether a part of the material which has become too turbid or too viscous for use can be recovered by filtration. It is also interesting and perhaps important to know what fraction of the protein is altered by denaturation. This can be determined to a reasonable degree of certainty by measuring the protein that can be recovered by filtration. With the small amounts of material available, it is not possible to determine the total amount held in the filter, and the concentration of albumin in the filtrate will depend upon the dryness of the filter pad before the filtration. The ratio of albumin concentration to that of sodium chloride should give the amount of protein held back without the corresponding amount of salt. We may assume that the solution entrapped in the meshes of the filter pad or of the unfilterable residue will contain the same ratio of albumin to salt as the filtrate.

A sample of standard commercial albumin, HA 35, was heated at 57° for 16 hours; the solute concentrations, turbidity, and viscosity were measured; it was filtered through a Seitz Ser. 3 filter; and the measurements were repeated. Another sample was heated 11 hours at 57°; the solute concentrations, turbidity, and viscosity were measured before and after 3 filtrations through Seitz Ser. 3 filters; and the stability of the filtered solution was measured nephelometrically and viscometrically. The results are given in Table VI.

The individual concentrations are changed only slightly and the ratio of albumin to salt indicates that no appreciable fraction of the protein is held back. Yet the nephelometer reading is reduced from 78 to 21 in the first case and from 36 to 13.5 in the second. The latter value is about as low as we achieved with small quantities of material. The filtration reduced the viscosity from 7.43 to 6.15 in the first case and from 6.88 to 6.37 in the second. However, the nephelometric and viscometric stabilities are both much smaller than for the unheated material. These results show that a very large part of the effect on turbidity and viscosity is due to a very small fraction of the material, and also that the stability of the filtered solution is much smaller than that of an unfiltered solution with the same turbidity or with the same viscosity. They give no indication whether this decreased stability is caused by a change in a small fraction of the molecules or by a smaller change in a large fraction of them.

CONCLUSIONS

Many of the results of these experiments have already been reflected in the production of

human serum albumin for the armed forces. The pH is specified at 6.8 ± 0.2 in order that the stability may be within 10 per cent of the maximum. The sodium chloride concentration now used, 0.3 M, lies between the concentration in plasma, 0.15 M, and 0.6 M, the concentration which would make the solution 0.15 M when diluted with water to have the same osmotic pressure as normal plasma. Stability considerations demand that the salt concentrations be as high as physiological requirements permit.

Although it is desirable that albumin solutions be kept in the cold, this does not appear important unless they are to be stored for a long time or are to be subjected at a later time to temperatures well above 100° F. (38° C.).

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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

V. THE INFLUENCE OF NON-POLAR ANIONS ON THE THERMAL STABILITY OF SERUM ALBUMIN [1,2,3]

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The present studies were initiated to increase still further the high stability that is characteristic of normal human serum albumin as prepared for the armed forces (1). The nephelometric and viscosimetric characterization of the standard solution has been discussed in detail in the preceding paper of this series (2).

There is the well-known solubilizing action on certain proteins of non-polar anions of comparatively high molecular weight. Some of these are the higher fatty acid anions; others are mixed aliphatic-aromatic anions of sulphonic acid. The latter are in common use as detergents. They not only solubilize many proteins but effect denaturation as well. The high temperature stability of serum albumin, however, was observed to be appreciably increased by the presence, in moderate concentration, of acetate ion. From this, it was considered that higher fatty acid anions would be deserving of study.

All of this work has been restricted to comparatively concentrated solutions of human serum albumin. The systems investigated are those described and characterized in the first, second, and fourth papers in this series (1 to 3). The conclusions drawn from the observations to be reported are not necessarily applicable to solutions of lower protein concentration.

The observations, furthermore, have been restricted to thermal stability at 50°, 57°, and at "cloud-point" temperatures. In the first 2 cases, we have applied conventional nephelometric technics with the modifications described in the fourth paper of this series (2), and designed to permit quantitative study of the formation of coagula or aggregates capable of producing a measurable change in the Tyndall effect. As has been pointed out (2), the method is sensitive; only a very small proportion of the total protein present contributes to the light scattering. The "cloud-point" method is also based on light scattering, but the proportion of total protein that participates in light scattering is higher than in the nephelometric method. Expressed differently, we may say that the end-points for the two methods are at very different levels.

METHOD

The nephelometric studies (50° and 57°) were carried out in the 15 cc. vials designed [4] for use in the Zeiss Nephelometer which is used in conjunction with the Pulfrich Photometer.

The cloud-point studies were carried out in thin-walled capillary tubes. The albumin solutions contained therein were heated at a constant and comparatively high temperature until a sharply discernible cloud formed in the solution,—that is until the cloud-point was reached. The body of coagulated protein forms as a haze or cloud rather than as a heavy coagulum, under the conditions of these experiments. If the conditions are optimum, such that the cloud-point time is in the 10 to 60 second range, quadruplicate specimens agree to within 2 or 3 seconds. In

[1] This work has been carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Stanford University.

[2] The products of plasma fractionation employed in this work were developed from blood, collected by the American Red Cross, by the Department of Physical Chemistry, Harvard Medical School, Boston, Massachusetts, under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[3] We are indebted to Dr. John Edsall for suggesting experiments with propionate, to Professor Linus Pauling for assistance in the interpretation of some of the findings in the present study, and to the Cutter Laboratories for much cordial cooperation.

[4] Lieutenant Commanders L. M. Woodruff and S. T. Gibson, Medical Corps, United States Naval Reserve, are responsible for the design of these vials.

most cases, the cloud of coagulated protein disappears at once if the tube be withdrawn from the bath within a second or two of attaining the end-point. Within limits, cloud formation is repeatedly reversible on repeated heating and cooling though the cloud-point time becomes progressively shorter. In application of the method, use is made of a small constant temperature bath with windows, a projector for illumination of the capillary tubes, and thermoregulation to ±0.03° C. A fully detailed description of the cloud-point technic, suitably illustrated, has been published elsewhere (6).

EXPERIMENTAL

We have used exclusively either crystallized human serum albumin or standard serum albumin, characterized in other papers of this series (1 to 5). Solutions were prepared by mixing in the dry state albumin and sodium carbonate, the latter in quantities sufficient to give almost the desired pH. Water was added and solution of the albumin effected at room temperature or at 0° C. A stock solution, 33.3 per cent albumin, was thus prepared. Seventy-five cc. portions of these stock solutions were diluted with appropriate volumes of 1.2M sodium chloride and 1.2M fatty acid sodium salt to give final volumes of 100 cc. The experimental solutions thus obtained contained 25 per cent albumin[5] and were 0.3M in total salt (exclusive of the small amount of added carbonate).

RESULTS

Before tabulating the cloud-point data, mention should be made of an important incidental observation. From cloud-point determinations on many different preparations, each one being studied at several different temperatures, it was found that a semilogarithmic plot of the data so obtained, logarithm C.P.[6] against temperature, gave rise to a family of straight lines. This observation permits one in each and every case to calculate the 30-second cloud-point temperature and also the 65° cloud-point time. For comparative purposes, it is extremely helpful to express the results for different preparations or for different experimental conditions in a similar way, preferably as the 30-second cloud-point temperature. Here we might interpolate a second observation which is of some physico-chemical interest: The slope of the curve, log C.P. against temperature, is a function of protein concentration, the pH, and the nature of the added salt. Within limits, it is independent of the concentration of the added salt (sodium chloride).

The salts studied were sodium chloride, sodium acetate, sodium propionate, sodium butyrate, sodium valerate, sodium caproate, sodium caprylate, sodium phenylacetate, and sodium phenylbutyrate,—usually in an over-all salt concentration of 0.3M. The effects upon high temperature thermal stability (cloud-point) are presented in Table I. Many other substances have been studied, but the results are of little, if any, promise: Succinate, fumarate, lactate, glucose, alanylglycine, glycerophosphate, and γ-globulin. It is of interest that the last named, in a concentration of 1.7 per cent, did not decrease the cloud-point of albumin in 0.3M chloride.

TABLE I

Increase of thermal stability of serum albumin by non-polar anions

Salt added	Concentration M	pH*	30-second C.P. temperature
Chloride	0.3	6.78	67.29
Acetate	0.3	6.88	68.02
Propionate	0.3	6.88	71.48
Butyrate	0.3	6.97	75.09
Valerate	0.3	6.75	78.40
Caproate	0.3	6.78	79.96
Caprylate	0.3	A clear translucent gel formed between 80° and 100°; no cloud formation.	
Phenylacetate	0.3	6.76	78.20
Chloride	0.15	7.26	63.1
Chloride Butyrate	0.15 0.15	7.28	71.7
Chloride Phenylacetate	0.15 0.15	6.84	76.9
Chloride Phenylbutyrate	0.15 0.15	7.15	81.5
Chloride Caprylate	0.15 0.15	6.68	81.8

* The values in this column are not for 25 per cent solutions of albumin but for solutions diluted 25-fold.

[5] The effect of protein concentration on the cloud-point is inverse. Thus, a decrease in albumin concentration from 45 per cent to 5 per cent increased the 65° cloud-point about 35-fold.

[6] Cloud-point is abbreviated as C.P.

The effects of the non-polar anions on 50° and 57° thermal stability are presented in Figures 1 and 2, respectively.

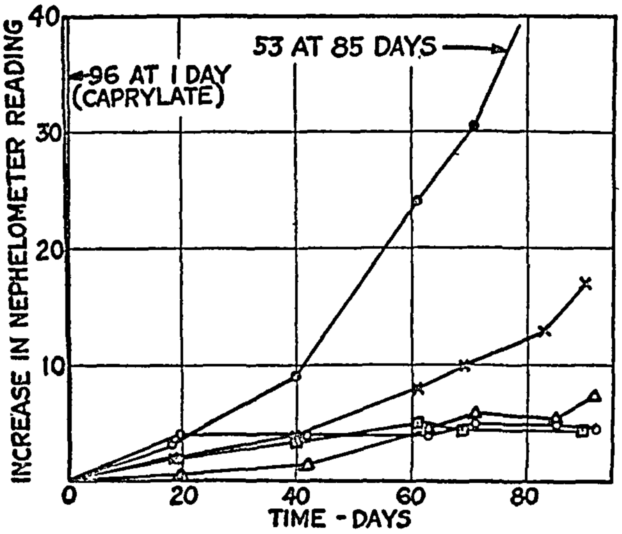

FIG. 1. EFFECT OF VARIOUS SALTS ON THE STABILITY OF PREPARATION 94–95 AT 50° C.

● 0.15M Chloride
× 0.15M Chloride and 0.15M Chloride
⊡ 0.15M Chloride and 0.15M Butyrate
△ 0.15M Chloride and 0.15M Phenylacetate
○ 0.15M Chloride and 0.15M Phenylbutyrate
⊠ 0.15M Chloride and 0.15M Caprylate

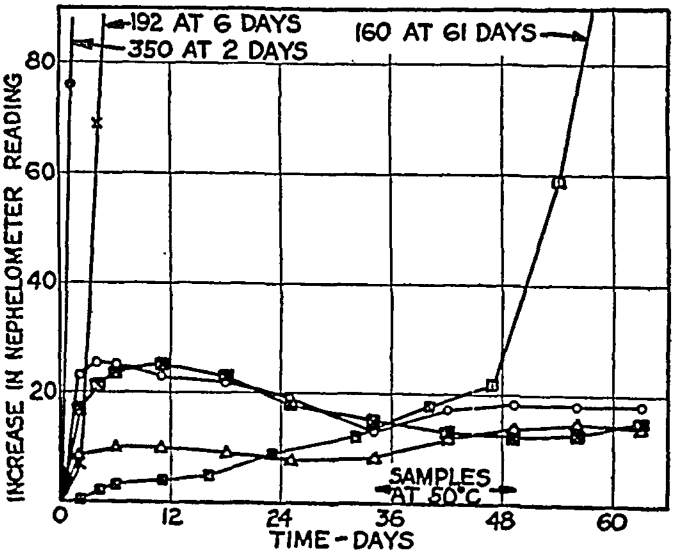

FIG. 2. EFFECT OF VARIOUS SALTS ON THE STABILITY OF PREPARATION 94–95 AT 57° C.

● 0.15M Chloride
× 0.15M Chloride and 0.15M Chloride
⊡ 0.15M Chloride and 0.15M Butyrate
△ 0.15M Chloride and 0.15M Phenylacetate
○ 0.15M Chloride and 0.15M Phenylbutyrate
⊠ 0.15M Chloride and 0.15M Caprylate

DISCUSSION

The marked stabilizing effect of the non-polar anions, studied on high temperature thermal stability (57° and cloud-point), are clearly evidenced by the results presented in Figure 2 and Table I, respectively. The experiments at 50° (Figure 1) are not yet terminated but are in qualitative agreement (except for caprylate) with the results observed at higher temperatures. With caprylate, the results are curious in that heat treatment (70°, 15 minutes) restores its stabilizing action as measured nephelometrically (at 50°). The increase in stability, conveyed by doubling the concentration of sodium chloride (Figures 1 and 2), confirms essentially the observations reported by Scatchard and co-workers, in the preceding paper of this series (2).

We are disposed to conclude that the added substances, in the comparatively high concentration used, do not inhibit denaturation of the protein molecule. The evidence now at hand, supported by some unpublished work, suggests that the albumin molecule begins to open out on heating and in doing so exposes more positively charged groups in side chains of amino acid residues. The stabilizing agent then becomes associated with the exposed amino groups ($R-NH_3^+$) through an electrostatic attraction with the carboxyl group of the added anion ($R'-COO^-$). The non-polar portion of the anion is attracted by the amino acid side chain in accordance with Van der Waals' forces; these, considered in the aggregate, would increase with increase in length of the carbon chain of the added substance. The protein-fatty acid anion complex must be regarded as possessing a greater solubility than denatured protein itself and of comparatively little tendency to flocculate out in coagula.

In so far as denaturation and coagulation are involved, we consider that interpretation of the phenomenon reported in this paper rests upon the theories of denaturation and coagulation now widely accepted among protein chemists and regarded as applicable to albumins particularly: an unfolding or opening out of the protein molecules in solution, followed, under certain conditions, by their flocculation, aggregation, or polymerization to give particles that are large

enough to scatter light or to form discrete coagula (7 to 18). This picture of denaturation as an opening out process is not applicable evidently to certain molecules such as myosin which in their native state are already quite extended (19).

SUMMARY

1. The thermal stability of serum albumin in 25 per cent solution has been studied at 50°, 57°, and cloud-point temperatures.

2. The capillary-tube cloud-point technic for studying protein coagulation is described.

3. Various non-polar anions are shown to enhance the thermal stability of human serum albumin, the effect increasing with increase in length of the fatty acid anion.

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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

VI. THE OSMOTIC PRESSURE OF PLASMA AND OF SERUM ALBUMIN[1,2]

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In order to determine the relative dosage of plasma and of serum albumin in the treatment of shock and to determine their efficiencies in increasing blood volume and other characteristics, we have measured the osmotic pressure of human plasma and serum, of regenerated solutions from dried plasma up to four-fold physiological concentrations, of human serum albumin solutions up to 300 grams per liter in 0.15 molar sodium chloride, and of bovine serum albumin over large ranges of pH, albumin concentration, and salt concentration.[3] We have not been able to detect a difference in the osmotic pressures of human and bovine albumin, so we have used the measurements on bovine albumin to extend the range of those on human albumin.

The first function of plasma or serum albumin in shock is to increase the blood volume by holding water in the blood stream. Parts of the water may be injected with the protein, may be shifted from the extravascular fluids, or may be drawn from the intestine. This water must be held in spite of the excess pressure in the capillary bed. Most of this retention of water is not caused by any attraction between the protein and the water, but arises from the fact that the chemical potential of water is decreased by the presence of dissolved molecules which are not water.[4]

A convenient way of measuring the change in the potential of water produced by the addition of a solute is to measure the decrease in pressure necessary to maintain equilibrium through a membrane permeable to the water but not to the solute, and this pressure is called the osmotic pressure of the solution. If the membrane is permeable to any of the solutes, the pressure is sometimes called the colloid osmotic pressure or

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[1] This work has been carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[2] This paper is Number 16 in the series "Studies on Plasma Proteins" from the Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross.

[3] All measurements were made with collodion membranes, which are impermeable to plasma proteins. For technical reasons, the measurements were made at 25° C., where the pressure is about 4 per cent less than at 37° C. All concentrations were calculated using the nitrogen factor 6.25 for both albumin and plasma.

[4] The difference in the potential of any substance at two different places is the least work necessary to bring unit quantity of that substance from the first place to the second if the temperature and total volume of the system are constant. The substance will shift spontaneously from the place of higher potential to that of lower potential if a path is available, and at equilibrium its potential must be the same throughout the system. The rapidity with which equilibrium is attained depends upon the nature of the available paths as well as upon the difference in potential, but the position of equilibrium depends only upon the equality of potential.

There may be a difference in gravitational potential due to different heights in a gravitational field, and this potential is proportional to the difference in height and to the weight of unit quantity. There may be a difference in chemical potential due to different pressures, and this potential is proportional to the pressure difference and to the volume of unit quantity. There may also be a difference in chemical potential due to different chemical compositions. We cannot generalize on the value of this potential, but in very dilute solutions, the difference in chemical potential of each substance is proportional to the difference in the logarithm of its mole fraction. Then the difference in the potential of each solute is proportional to the difference in the logarithm of its concentration, and the difference in potential of the solvent is proportional to the difference in the sum of the concentrations of the solutes, expressed as moles of solute per unit quantity of solvent. These are the three potentials of substances which are physiologically important.

the oncotic pressure. Since this is the only type of membrane across which the pressure is important, we will use the simpler term.

Van't Hoff pressure

About two-thirds of the osmotic pressure at physiological concentrations is explained by the simple theory of Van't Hoff, which says that the osmotic pressure is equal to the pressure difference which would be developed if the solvent were removed and the solutes were gases. If the non-diffusible solute is a non-electrolyte, the osmotic pressure should be proportional to its concentration, and the pressure-concentration ratio should be the same for all substances if the concentration is expressed as moles per unit volume. It is obvious that osmotic pressure per unit mass, however, decreases as the size of the molecule increases. If the concentration is expressed as mass per unit volume, the pressure-concentration ratio is inversely proportional to the molecular weight, and one measurement of the osmotic pressure and of the corresponding mass concentration may be used to determine the molecular weight. The Van't Hoff case is illustrated by curve E in Figures 1 and 2. In Figure 1, the osmotic pressure is plotted against the concentration, and Van't Hoff's theory leads to a straight line whose slope is inversely proportional to the molecular weight. In Figure 2, the pressure-concentration ratio is plotted against the pressure, and this curve E is a horizontal line whose ordinate is the slope of E in Figure 1. The curves A in these figures are the measured values for serum albumin at pH 7.4, and the curves B are for albumin at pH 5.4, both in 0.15 M sodium chloride. At pH 5.4, the albumin is iso-ionic; that is, its average net charge is zero.

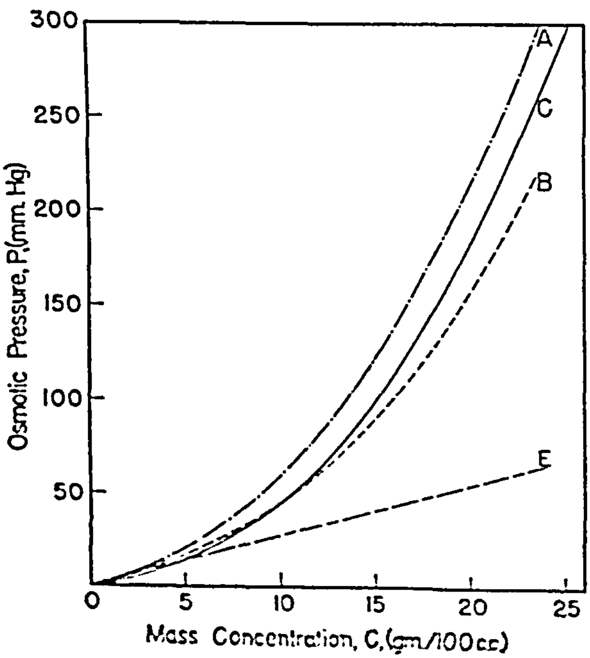

FIG. 1

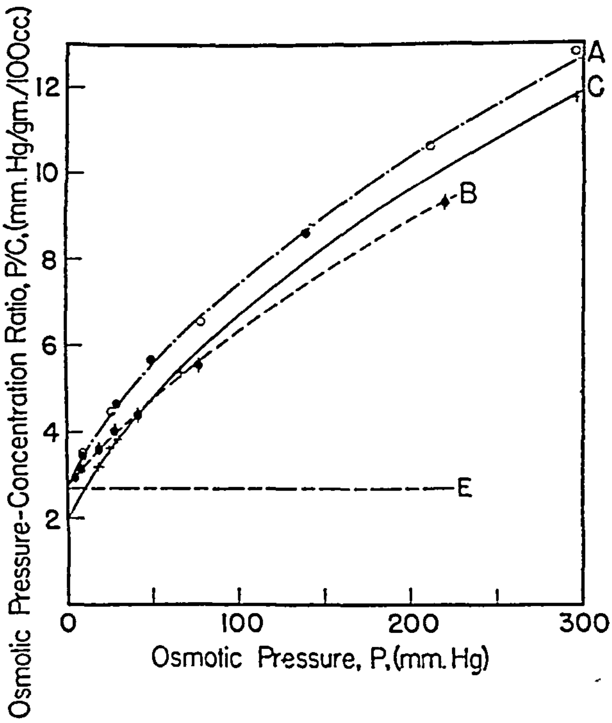

FIG. 2

Donnan pressure

The plasma proteins are not neutral non-electrolytes, and their ionic charges have an important effect on the osmotic pressure. The extension of Van't Hoff's theory to this case was carried out by Donnan. We will limit ourselves to the special case of the sodium salt of a non-diffusible protein anion and sodium chloride, which is a rough approximation of plasma. The solution must be electrically neutral. So, if there is no sodium chloride present, the pressure-concentration ratio corresponds to

the protein ion plus all the sodium ions associated with it.

If there is sodium chloride present, its potential must also be the same on the two sides. Since there is more sodium, there will be less chlorine on the side with the sodium proteinate. So the pressure-concentration ratio is smaller. It corresponds to the protein alone when the ratio of protein to chloride is very small, and depends only upon the protein-chloride ratio. The molecular weight cannot be determined from a single osmotic pressure measurement. However, if a series of measurements is made with varying protein concentration but constant salt concentration, the pressure-concentration ratio can be extrapolated to zero protein concentration, and the molecular weight can be determined from this extrapolated value. At pH 5.4, the Donnan curve is the same as the Van't Hoff curve E. The Donnan curve for pH 7.4, where the measured curve is A, lies close to the curve B. It is obvious that the Donnan effect increases rapidly with the net charge of the protein ion. It is, in fact, proportional to the square of the net charge.[5]

Pressure of real solutions

The Van't Hoff theory assumes that each individual water molecule in a protein solution behaves like a water molecule in pure water and that the decrease in potential of the water which

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[5] The statement in mathematical equations is more precise and in some ways simpler. We will let C, C_+ and C_- be the concentrations in moles per unit volume of protein, sodium ion, and chloride ion within the membrane, and C'_+ and C'_- the concentrations of sodium and chloride ions outside: c will be the concentration of the protein in grams per unit volume, M its molecular weight, which is c/C, z its valence (a negative number for an anion), T will be the absolute temperature, and R a universal constant.

Van't Hoff's law is

$$P = RT[C + C_+ + C_- - C'_+ - C'_-]$$

$$\frac{P}{c} = \frac{RT}{M}\left[1 + \frac{C_+ + C_- - C'_+ - C'_-}{C}\right]$$

The law of electroneutrality requires that

$$zC - C_- + C_+ = 0$$

and

$$C'_+ - C'_- = 0$$

The law of equilibrium, or equality of the potential of sodium chloride, requires that

$$C'_+C'_- = C_+C_-$$

Combination of the last three equations yields

$$C'_- = C_-\sqrt{1 - \frac{zC}{C_-}}$$

Combination with Van't Hoff's law yields

$$\frac{P}{c} = \frac{RT}{M}\left[1 + \frac{2C_- - zC - 2C_-\sqrt{1 - \frac{zC}{C_-}}}{C}\right]$$

$$= \frac{RT}{M}\left[1 + \frac{C_-}{C}\left(2 - \frac{zC}{C_-} - 2\sqrt{1 - \frac{zC}{C_-}}\right)\right]$$

$$= \frac{RT}{M}\left[1 + \frac{C_-}{C}\left(1 - \sqrt{1 - \frac{zC}{C_-}}\right)^2\right]$$

It is clear from the last equation that the expression in square brackets depends only on zC/C_- for a given value of z, but it is not clear how it approaches unity as zC/C_- approaches zero or even that it does so. To answer these questions, we expand the radical in a Taylor's series to give

$$\sqrt{1 - \frac{zC}{C_-}} = 1 - \frac{zC}{2C_-} - \frac{z^2C^2}{8C_-^2} \cdots$$

$$\frac{P}{c} = \frac{RT}{M}\left[1 + \frac{z^2C}{4C_-} + \cdots\right]$$

$$= \frac{RT}{M} + \frac{RT(z/M)^2c}{4C_-} = \frac{RT}{M} + \frac{M(z/M)^2P}{4C_-}$$

For a neutral molecule, z is equal to zero, and $P/c = RT/M$ at all concentrations. For an ion $z^2C/4C_-$ is zero only when C is zero, and in dilute solutions P/c increases as a linear function of c when C_- is constant.

For a real solution we may also write

$$\frac{P}{c} = \frac{RT}{M} + bc + dc^2 + \cdots$$

or

$$\frac{P}{c} = \frac{RT}{M} + BP + DP^2 + \cdots$$

in which b and d or B and D are constants. The latter form is more useful for our purposes because

$$\frac{1}{c} = \frac{RT}{MP} + B + DP + \cdots$$

In many cases, D is so small that the term DP may be neglected. Then the volume of solution per gram of protein is equal to the ideal term which is inversely proportional to the pressure plus a term which is independent of the pressure.

B will be composed in general of the Donnan term which is $M(z/M)^2P/4C_-$ plus a term arising from the difference between the force between two protein molecules and that between one of the molecules and the water displaced by the other.

accounts for the osmotic pressure is solely a statistical effect, due to the fact that a certain fraction of the molecules are not water. This is equivalent to assuming that each individual protein molecule in a concentrated solution behaves like a protein molecule in an extremely dilute solution, or that the activity of the protein is proportional to its concentration, and this latter assumption is easier to consider. The Donnan extension considers the macroscopic effect of the charge on the protein ion, but still assumes that the activity of each species is proportional to its concentration.

Real protein solutions are not this simple.[6] For iso-ionic albumin, the osmotic pressure is much larger than that calculated by the Van't Hoff theory. At other values of pH, however, the increase over the iso-ionic value is much less than predicted by Donnan, so that at pH 7.4, the measured value does not differ greatly from that calculated by his theory, and at still higher values of the pH, the measured pressures are less than those calculated. Real solutions do resemble the Donnan solutions in that the molecular weight can be determined by extrapolating the pressure-concentration ratio to zero concentration or pressure.

Figure 1 shows the curves for osmotic pressure versus mass concentration for serum albumin at pH 7.4 (A) and pH 5.4 (B), for human plasma at pH 7.4 (C), and for an ideal Van't Hoff solute whose molecular weight is 69,000, which is the same as that of albumin (E). The curves all start at zero concentration and pressure, and curves A, B, and E, all have the same initial slope. The value for the molecular weight of serum albumin calculated from these osmotic pressure measurements, 69,000, is in good agreement with that obtained from sedimentation and diffusion measurements reported in the first paper of this series (1). The plasma curve (C) has a smaller initial slope which corresponds to a larger molecular weight, 93,000. All the experimental curves become steeper at higher concentrations. The difference between A and C is proportional to the concentration.

In Figure 2 is shown the pressure-concentration ratio, P/c, versus the pressure.[7] The ideal Van't Hoff curve, E, is here a horizontal line whose ordinate is the slope of E in Figure 1. A and B intercept E at zero pressure, corresponding to the same initial slopes in Figure 1, but they deviate sharply at higher pressures. The plasma curve, C, has a lower intercept, corresponding to the larger average molecular weight of plasma proteins, and the difference between A and C is here independent of the pressure. If 60 grams per cent of the plasma protein is albumin (1), curve C as drawn would give an average molecular weight of 194,000 for the remaining 40 per cent. However, our measurements do not preclude drawing the intercept at 2.1 rather than 2.0, which would correspond to an average molecular weight of 88,000, and a molecular weight of 150,000 for the 40 grams per cent which are not albumin. The probable value for the average molecular weight of plasma globulins is 170,000.

Osmotic efficiency

We are particularly interested in the volume of solution per gram of protein, $1/c$, at a given

[6] The ionic charges on proteins cause deviations from random distribution on the microscopic scale as well as those considered by Donnan, and differences in potential proportional to the square of the net ionic charge. Even in iso-ionic albumin, where the average of the charge is zero, the average of the square of the charge is 6, and at pH 7.4 it is 300. In either solution, the total charge is almost 200, and it is not evenly distributed through the molecule. The resultant electrical field leads to attraction between a protein molecule and a simple ion, or between 2 protein molecules, which is large compared to the effect of the net charges. Moreover, the protein molecule which bears these charges behaves more like an equal volume of a normal organic liquid than an equal volume of water. This molecular framework tends to repel the electrical fields but to attract another framework. The resultant of all these forces leads to effects upon the potential of the water and the resultant osmotic pressure which vary greatly with the ionic charge.

[7] The experimental points are shown in Figure 2, whose scale is larger than that of Figure 1. The measurements on plasma are indicated by crosses, those on human albumin by open circles, and those on bovine albumin by filled circles. The measurements at pH 5.4 have a perpendicular line through the circles.

The measurements on albumin at pH 5.4 are carried to dilute enough solutions so that there is little uncertainty about the extrapolation to zero concentration and the resultant molecular weight. The measurements on plasma have not been carried to such dilution, so the curve has been drawn parallel to that for albumin.

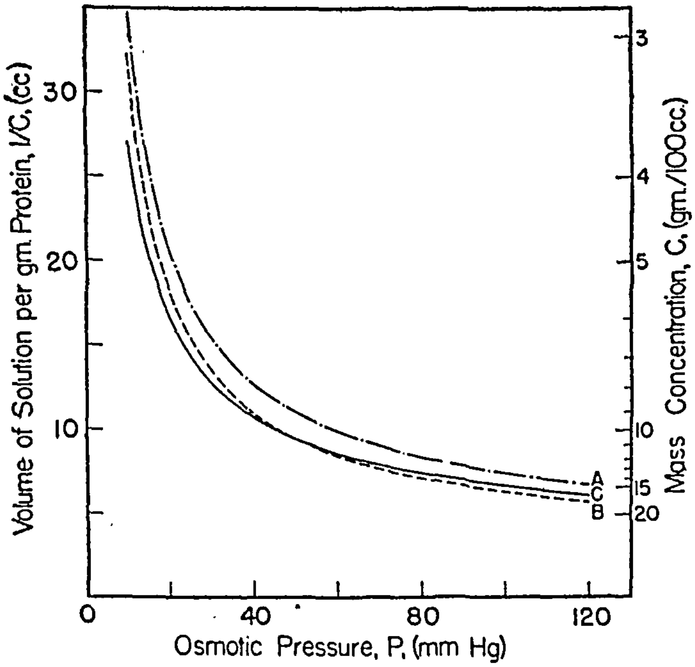

Fig. 3

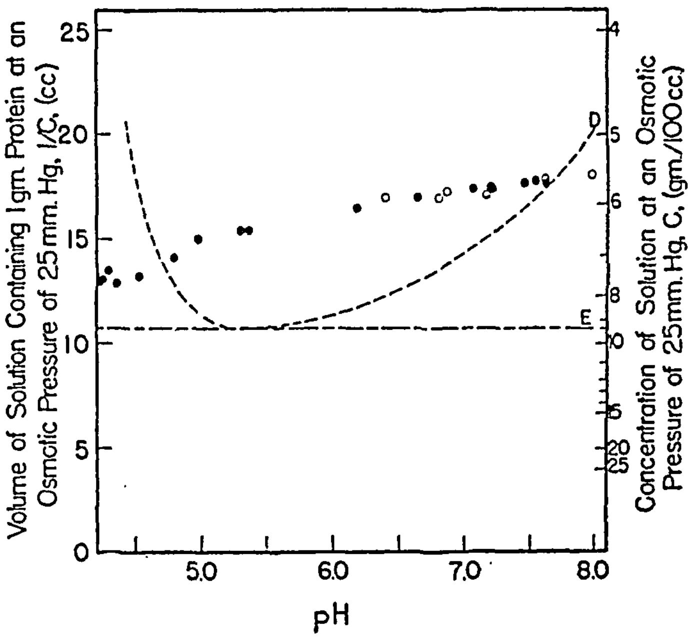

Fig. 4

osmotic pressure. This is shown in Figure 3 for the same measurements.[8] As in the previous figures, curves A and B refer to albumin at pH 7.4 and 5.4, respectively, and curve C to plasma at pH 7.4. The curve for an ideal Van't Hoff solute corresponding to E of Figures 1 and 2 is omitted. It would lie about 5 cc. below curve B, or about 7 cc. below A at all values of the pressure, as these three curves are nearly parallel. The difference between A and C is almost inversely proportional to the pressure.

Figure 4 shows the volume of solution per gram of protein at 25 mm. pressure as a function of the pH. The curve E represents as before the ideal Van't Hoff solute, with a molecular weight of 69,000, and the curve D represents an ideal Donnan solute with the same molecular weight and the net charge of albumin.[9] Although the difference between these curves and the experimental values is not very great at the physiological pH, it is evident that this is largely a coincidence, for the experimental and ideal curves cross at large angles.

[8] Figure 3 is constructed from the same data as Figures 1 and 2 except that the measurements at very high and very low pressures are both omitted to permit a larger scale. The individual points are again omitted.

[9] The titration curve for human albumin lies somewhat to the left of the curve for bovine albumin, used in estimating net charge for these calculations.

The experimental values of the volume per gram of protein, $1/c$, between pH 6 and 8 may be expressed by the straight line

$$1/c = 11.1 + 0.9\ \mathrm{pH}$$

Since the difference between $1/c$ and the Van't Hoff value RT/MP is nearly independent of the pressure, a good approximation for $1/c$ at any small pressure is

$$1/c = 268/P + 0.4 + 0.9\ \mathrm{pH}$$

This corresponds to

$$P/c = 268 + (0.4 + 0.9\ \mathrm{pH})P = \frac{268}{1 + (0.4 + 0.9\ \mathrm{pH})c}$$

COMMENT

The chief uncertainty in determining the efficiency of albumin in increasing blood volume lies in the uncertainty of the osmotic pressure of normal plasma, which goes back to the uncertainty as to the normal concentration of plasma proteins. The composition of the proteins appears to be very constant in physiological plasma (1),[10] but the total concentrations quoted by

[10] Wies and Peters (2) have studied the effect on the osmotic pressure of varying composition of the protein in pathological plasma. Their results cannot be compared with ours directly because they determine proteins by Howe's precipitation method and our compositions are determined by electrophoresis. If we assume with them that

various observers show variations. Gutman and his coworkers (3) quote protein concentrations in serum for normal adults ranging from 6.5 to 7.9 and averaging 7.2 grams per cent. Perera and Berliner (4) quote concentrations from 6.2 to 7.3, averaging 6.8 for normal ambulatory adults, and from 5.4 to 7.0, averaging 6.0 for the same individuals recumbent. The results for plasma should be 0.4 per cent higher than for serum. So we must consider the range from 6 to 8 grams per cent. Since the recipient of a blood substitute will probably be resting, the lower part of the range is probably more important than the higher.

If c is 6 grams per cent, $1/c$ is 16.7 cc. per gram. From curve C, Figure 3, we find the corresponding pressure to be 20 mm., and from curve A we find that at 20 mm. pressure, each gram of albumin retains 20 cc. of solution. If c is 8 grams per cent, $1/c$ is 12.5 cc. per gram. The corresponding pressure is 32 mm., and at that pressure, each gram of albumin retains 15 cc. of solution. If the pressure is 25 mm., each gram of albumin retains 18 cc. of solution, and each gram of average plasma protein retains 15 cc., or the concentration of plasma protein is 6.67 grams per cent. This is the value which we have taken as the norm, with the realization that it may vary more than 10 per cent in normal individuals. This corresponds closely to the average of 17.4 cc. per gram of albumin, given for the increase in blood volume per gram of added albumin by Heyl, Gibson, and Janeway (5, 6). This average corresponds to 6.9 per cent protein. Their extreme values of 13.2 and 24 cc. per gram correspond to 5.1 and 8.8 per cent protein. Probably part of this variation corresponds to the inevitable errors of measurements of total blood volumes.

If the increase of blood volume on the addition of protein is to equal the volume of solution retained by the protein, the osmotic pressure of the plasma must be the same after the addition of the protein as before. It is probable that this condition is nearly fulfilled in hemorrhagic shock and also in traumatic shock. Any error in this assumption will be in the direction of increasing the pressure during the infusion and thus giving a smaller increase in blood volume. It is possible, however, that there are cases in which the assumption is so greatly in error that it is more accurate to assume that the blood volume is constant, and to determine the increase in osmotic pressure per gram of protein at constant plasma volume. Thus, an increase in osmotic pressure per gram of protein at constant volume becomes of interest. This will, of course, depend upon the total plasma volume, of which we have no measure here. Our measurements do show the change in pressure for unit change in concentration, and most clearly in Figure 1. This change, dP/dc, for albumin is 2.7 at zero concentration, 3.9 when c is 2, 5.1 when c is 4, 6.3 when c is 6, and 8.4 when c is 8 grams per cent. The values of dP/dc for average plasma protein at the same concentrations are 2.0, 2.8, 3.8, 4.0, and 6.4. For albumin or for plasma, the change in pressure with changing concentration increases rapidly as the concentration increases.

The comparison of the relative efficiencies of serum albumin and of plasma is more certain. Within the accuracy with which we read our curves, the relative efficiencies are independent of the pressure. There is a slight increase in the relative efficiency of albumin as the pressure decreases, but we may take, for all physiological pressures, our result that each gram of albumin retains as much fluid as 1.2 grams of average plasma protein. The pooling of plasma from several donors and the relative constancy of conditions of collecting blood reduce the fluctuations in the concentration of plasma. At present, the Red Cross pooled citrated plasma contains 6 grams per cent of protein (1). So each gram of albumin corresponds to 20 cc. of citrated plasma and 25 grams of albumin corresponds to 500 cc. of citrated plasma. This relationship is the basis for the present containers used by our armed forces,—500 cc. of citrated plasma for the

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the pressure is a linear function of the ratio of globulin to total protein, g, at all small pressures and pH's between 6 and 8, we may determine the effect of globulin from our measurements on normal plasma, which gives

$$P/c = \frac{268(1 - 0.42\,g)}{1 + (0.4 + 0.9\,\mathrm{pH})c}$$

For normal plasma this equation gives a limiting value at zero concentration which is too large, but it should give a fair representation of the results in undiluted plasma or serum.

dried plasma container or 100 cc. of 25 per cent albumin.

SUMMARY

The osmotic pressures of plasma and of serum albumin at 25° have been measured over ranges of concentration and pH, much wider than the physiological ranges. The extrapolation of the osmotic pressure-concentration ratios to zero concentration yields a molecular weight of 69,000 for albumin. A similar extrapolation for plasma yields an *average* molecular weight of about 90,000. This corresponds to an *average* weight of about 170,000 for the 40 per cent of the protein which is not albumin. The osmotic pressure–concentration ratios increase rapidly with increasing concentration. At pH 7.4, this increase corresponds to the Donnan effect of the ionic charges, but measurements over a pH range show this to be a coincidence.

The volume of fluid held in the blood stream by each gram of albumin should be about 18 cc. but should vary with the protein concentration of the plasma. Each gram of albumin is equivalent to 1.2 grams of plasma protein or 20 cc. of the current Red Cross citrated, pooled plasma.

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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

VII. CONCENTRATED HUMAN SERUM ALBUMIN [1,2,3]

PART I. ALBUMIN IN THE TREATMENT OF SHOCK.
PART II. SAFETY OF ALBUMIN.
PART III. ALBUMIN IN THE TREATMENT OF HYPOPROTEINEMIA.

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INTRODUCTION

Specialized combat units, such as landing parties, paratroops, seabees, and commandos, require fighting equipment and supplies especially adapted to their needs. All supplies should be compact, as transportation space is at a premium in these highly mobile units. The Standard Army and Navy Package of Dried Plasma is bulky, and therefore concentrated human serum albumin was developed (1) to meet the needs of such groups for a concentrated blood derivative.

Although albumin may not be available in civilian practice for some time, this paper summarizes our experience in the hope that it may serve as the starting point for future studies. This experience has been gained over a two-and-a-half-year period, the first part of which was devoted to an evaluation of albumin as a therapeutic agent in shock,[7] and the latter part to the testing of each albumin lot for acceptance by the armed forces.

It must be emphasized that the work to be presented represents the cooperative efforts of so many individuals that it would be impossible to name all those whose labors have gone into these studies. Credit must go to the American Red Cross Blood Donor Service, which organized the collection of the blood, from which both human plasma and human serum albumin have been prepared, and to the laboratory workers who carried on the production of albumin during the experimental stages of the program. The large-scale production which has been accomplished was made possible through the development of sound theoretical procedures by those working in the laboratory where the methods originated.

PART I. ALBUMIN IN THE TREATMENT OF SHOCK

A. The theoretical and experimental basis for its use

Serum albumin has at least two known functions: it maintains the colloid osmotic pressure of the blood and plays a rôle in the nutrition of the tissues. We are primarily concerned with

[1] This work has been carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[2] This paper is Number 17 in the series "Studies on the Plasma Proteins" from the Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross.

[3] This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the United States Navy. The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting views of the Navy Department or the Naval Service at large.

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[6] Captain, Medical Corps, United States Navy.

[7] The appraisal of albumin was aided greatly by Soma Weiss, R. F. Loeb, A. Blalock, I. S. Ravdin, and O. H. Wangensteen.

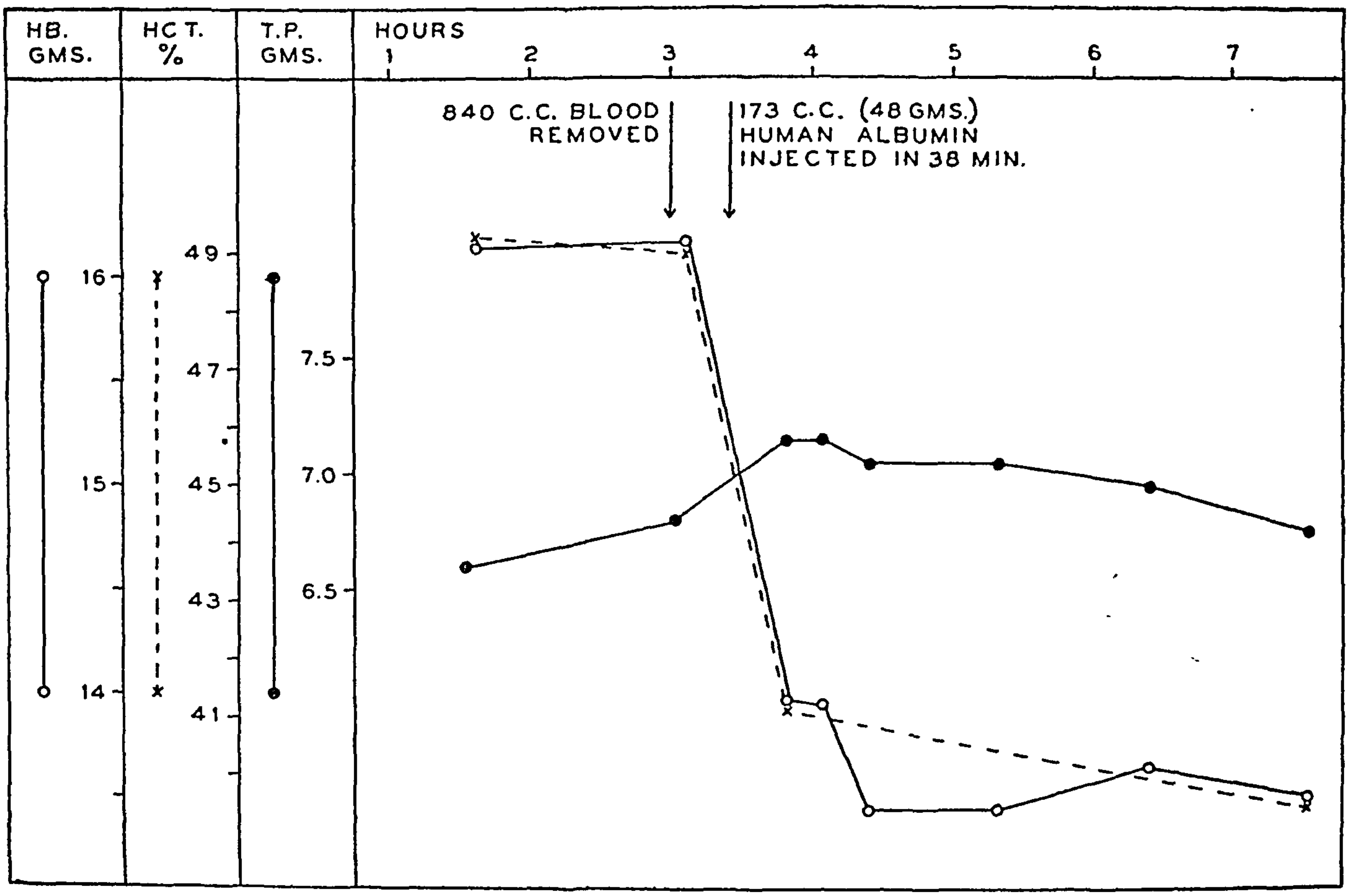

FIG. 1. RESPONSE OF A NORMAL HUMAN SUBJECT TO VENESECTION FOLLOWED BY RAPID INTRAVENOUS INFUSION OF CONCENTRATED HUMAN ALBUMIN
(data of Stead and Ebert)
(Reproduced from Mudd and Thalhimer's "Blood Substitutes and Blood Transfusion" with the permission of the publisher, Charles C. Thomas)

the former in this section. Although it comprises but about 60 per cent of the plasma proteins, albumin accounts for approximately 80 per cent of their colloid osmotic pressure (2). This high osmotic activity makes serum albumin an important factor in the regulation of the volume of circulating blood.

Certain properties of serum albumin have much to do with its practical therapeutic usefulness. Not only is it osmotically active, but also extremely stable, and very soluble in water or in solutions of various crystalloids. Dispensed as a concentrated solution, albumin takes up much less space than a comparable amount of dried plasma and is ready for instant injection without reconstitution. As with plasma, no cross-matching is necessary. Its stability is an important asset for military use, permitting transportation over long distances without refrigeration. The relative symmetry of the albumin molecule imparts a low viscosity to albumin solutions; in fact, a 25 per cent solution is approximately isoviscous with whole blood (2).

The proposal to use concentrated albumin in the treatment of shock depended on the premise that, due to its high colloid osmotic pressure, it would increase the circulating blood volume by drawing on the tissue fluids of the patient. Preliminary experiments in human subjects by Stead and Ebert (3) showed that this actually was the case. Measurements made by Scatchard, Batchelder, and Brown (4) indicated that each gram of albumin should hold 18 cc. of fluid in the circulation by virtue of its colloid osmotic pressure. In conjunction with their studies, some of us (5) made measurements of the plasma volume increase produced by albumin injection following rapid blood depletion by venesection. The average increase (one hour after injection) was 17.4 cc. per

gram, although there was considerable variation between individual experiments. This corresponded closely with the 18 cc. expected and justified the belief that concentrated albumin would exhibit the same type of osmotic behavior *in vivo* as *in vitro*. Consequently, 25 grams of albumin, representing the osmotic equivalent of 500 cc. of citrated plasma, was taken as the standard dose.

The immediate response of patients to an injection of concentrated albumin is quite uniform. There is a rapid fall in hemoglobin concentration and hematocrit reading, indicating the transfer of extravascular fluid into the circulation. Unlike the hemodilution due to an injection of saline solution, that following albumin is not accompanied by a fall in the serum protein concentration. In Figure 1, the response of a patient to concentrated albumin after blood loss is shown. This illustrates the rapid hemodilution which occurs, associated with a slight increase in serum protein concentration. Most of the effect of the albumin is exerted during the period of injection, but for the next few hours, there is a gradual decline in the values for hemoglobin and hematocrit, with corresponding return of the serum protein value to normal. Figure 2 illustrates the difference in hematocrit response after acute blood loss following the injection of concentrated albumin or of saline solution, as compared to the changes occurring in an untreated control subject.

When concentrated albumin is injected into a patient with a normal blood volume, a similar immediate hemodilution occurs. However, in such a patient the hemodilution is transient, and the values for hemoglobin and hematocrit return toward normal in a few hours. This contrast in response between patients with normal and depleted blood volumes is shown in

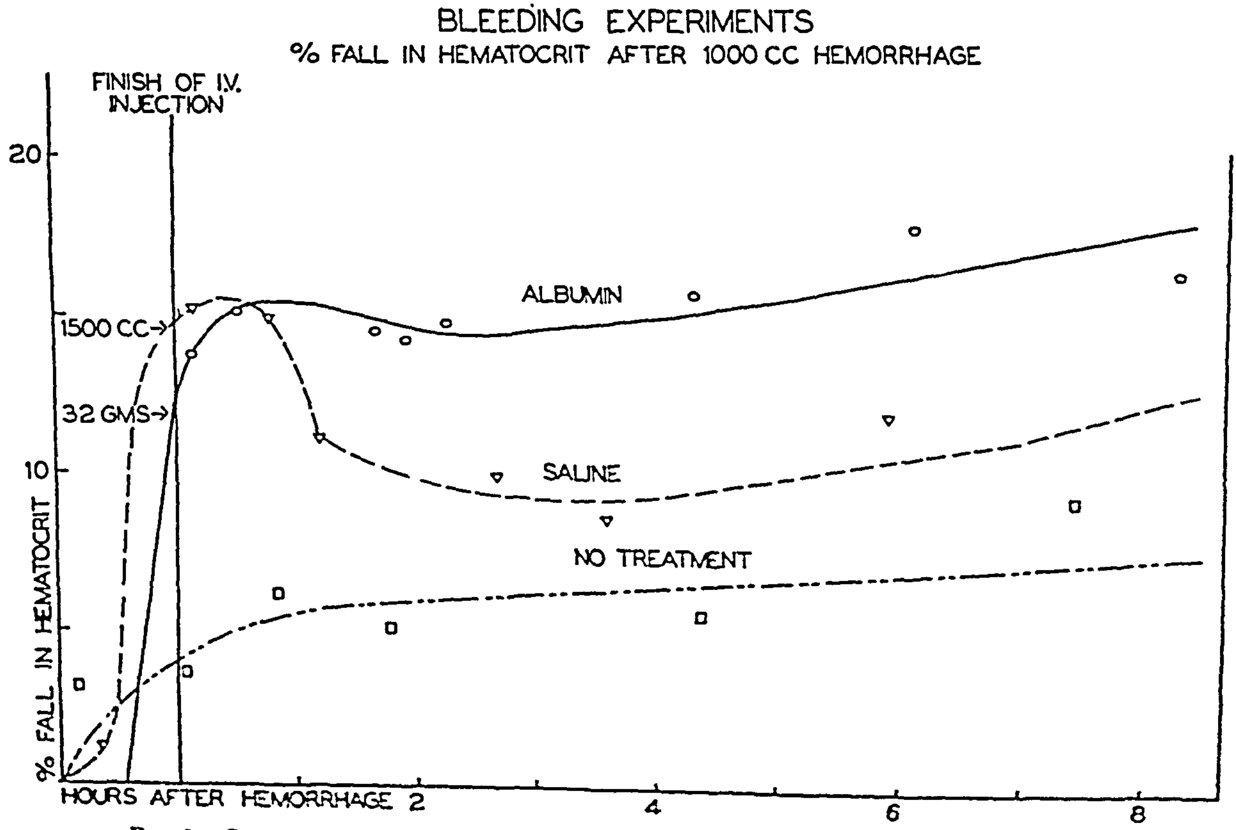

FIG. 2. CHANGE IN HEMATOCRIT READING OF HUMAN SUBJECTS AFTER ACUTE BLOOD LOSS

Note sustained effect of albumin contrasted with transient effect of saline. From data of Ebert, Stead, and Gibson (29) and Heyl, Gibson, and Janeway (5).

(Reproduced from the *U. S. Naval Medical Bulletin* with permission of the Editor)

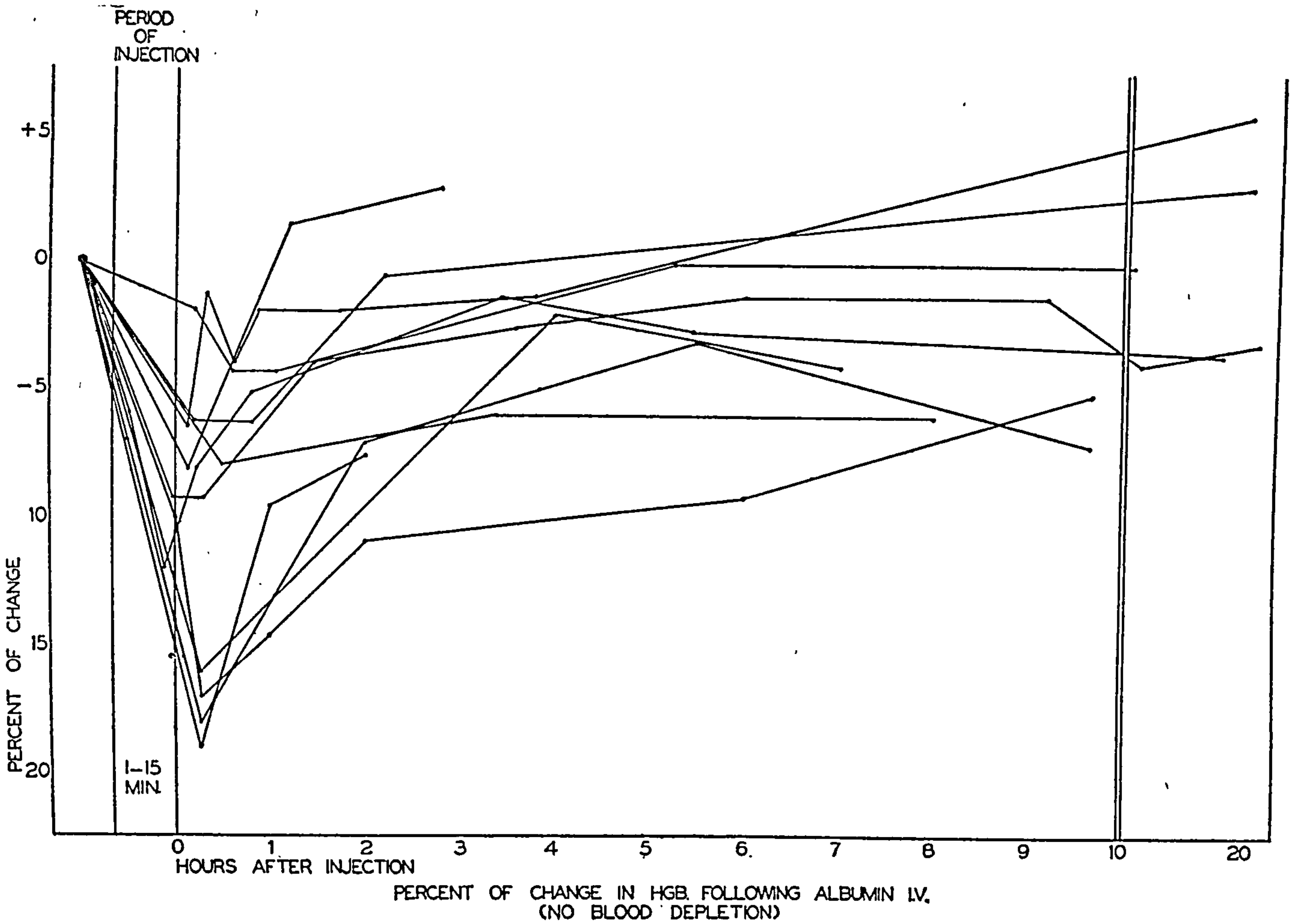

FIG. 3. CHANGE IN HEMOGLOBIN CONCENTRATION AFTER INJECTION OF 25 GRAM DOSES OF CONCENTRATED HUMAN ALBUMIN IN SUBJECTS WITH NORMAL BLOOD VOLUMES

Figures 3 and 4, and is in agreement with the results obtained by Sharpey-Schafer and Wallace with serum (6).

B. Clinical evaluation

The experimental evidence cited above seemed to confirm so well the anticipated theoretical advantages of concentrated albumin that a clinical program was instituted to determine whether the value of albumin could be proved in a series of actual cases of shock. To get a rapid, impartial evaluation, the cooperation of a number of clinics [8] throughout the United States was obtained, and their results were submitted to us for study. At the outset, it was felt that 1000 cases of shock of various types should be treated for the proper evaluation of this material. However, when the first 200 patients had been treated, of whom only 75 were suffering from shock and 25 from burns, and the results analyzed and reported to the appraisal committee, it was their opinion that the value of albumin had been demonstrated, so that no further delay was warranted before recommending it to the Army and Navy. It was also felt that their needs were so urgent that no more albumin should be used for experimental purposes. However, small amounts of albumin subsequently have been allocated to two groups of investigators, whose detailed studies of selected shock cases might contribute information of value to the armed forces. The results of these investigations are presented in subsequent papers in this series (7, 8). The results in the original

[8] Atlanta, Ga., Grady Hospital; Baltimore, Md., Johns Hopkins Hospital; Boston, Mass., Beth Israel Hospital, Boston City Hospital, Massachusetts General Hospital, Peter Bent Brigham Hospital; Chicago, Ill., Michael Reese Hospital; Iowa City, Iowa, University Hospital; New York, N. Y., Memorial Hospital, Presbyterian Hospital; Philadelphia, Pa., University Hospital; Washington, D. C., United States Naval Hospital, Walter Reed Hospital.

group of 200 cases have been previously reported (9).

1. *Methods of study*

The clinical program was set up in an attempt to test the effect of varying doses of albumin in the treatment of different types of shock and to collect all data possible on the advantages and disadvantages of this material. Emphasis was placed upon attempting to demonstrate an increase in circulating blood volume, such as had been observed in experimental subjects. Since it was not feasible to determine the plasma volume in these cases, it was decided to rely upon changes in the hematocrit reading as an index of changes in the plasma volume.

Case reports were made on special forms sent to all cooperating physicians. The data on these were carefully studied in preparing a report for the appraisal committee. That report (9) and the present paper are based on this analysis of cases, treated in cooperating clinics, and on personal observation of most of the cases treated in Boston hospitals, where two of us were on call at all times and carried on the evaluation under the helpful guidance of Dr. Soma Weiss.

Because of the small number of cases, a statistical analysis would be of little significance, particularly in view of the variation in types of cases. Reliance could not be placed entirely on laboratory data, because it was necessarily inadequate in many instances. In cases which showed marked clinical improvement without change in the recorded hematocrits, the results were considered satisfactory, particularly in those patients who received saline before treatment with albumin was instituted. In some instances, the physician did not withhold plasma or blood indefinitely. Such cases often proved particularly instructive because of this other treatment as, for example, those showing little or only temporary response to saline, but marked and sustained improvement with albumin. Likewise, cases showing little response to albumin and later no further response to whole blood transfusion, made a more accurate interpretation of the results possible. The administration of additional fluids prevented an evaluation of the effects of albumin in dehydrated patients, but since all the traumatic shock cases were treated soon after injury, none was sufficiently dehydrated to have provided information on this point.

2. *Results*

The cases of shock have been classified into several groups: traumatic, hemorrhagic, operative, and shock associated with infection. Cases of traumatic shock often overlapped those of hemorrhagic shock, and such cases were classified according to which seemed to be the more important factor. Each group of cases was analyzed with respect to the number showing improvement in general condition, hematocrit

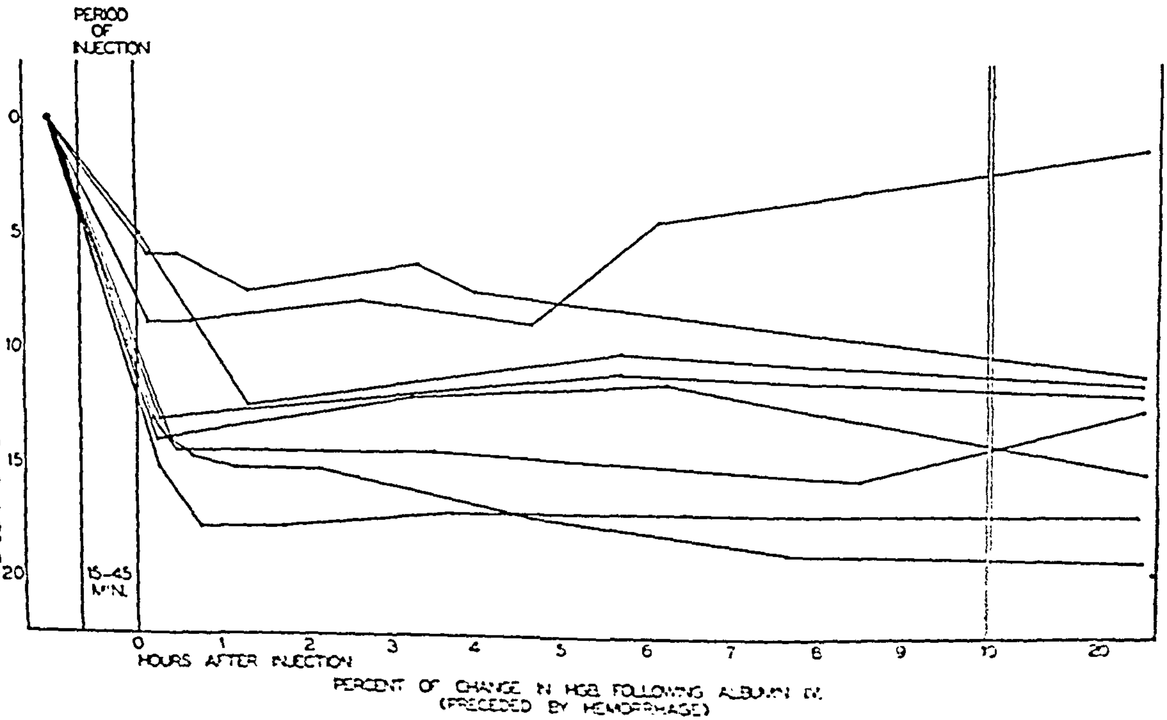

FIG. 4. CHANGES IN HEMOGLOBIN CONCENTRATION AFTER INJECTION OF VARYING DOSES OF CONCENTRATED HUMAN SERUM ALBUMIN IN SUBJECTS WITH BLOOD VOLUMES DEPLETED BY HEMORRHAGE

TABLE I

Selected shock cases

Diagnosis	Clinical condition			Blood pressure		Pulse		Hematocrit		Other fluids		Remarks
	Before treatment	After treatment		Before	After	Before	After	Before	After	Before	After	
		½ hour	1 to 2 hours									
A. TRAUMATIC SHOCK												
Compound fracture, skull and humerus	Mild shock	Much improved	B.P. still elevated	70/40	140/80	120				0	0.25 L. blood 2 to 3 hours later	Prompt restoration of B.P. with improved clinical condition allowing skull repair.
Fractured femur	Mild shock	Definitely improved	Stabilized	80/50	100/60	120	106	23	23	1 L. saline	0	B.P. promptly restored and maintained in contrast to previous saline response which was not maintained.
Compound fracture, humerus	Severe shock	Improved	Good for 3 hours	Unobtainable	158/60	No pulse	76				10 per cent D in S; blood at 3 hours	First dose gave prompt improvement. Blood transfusion at 3 hours. Relapse at 8 hours.
Same case 8 hours later	Severe shock	Improved	B.P. maintained	Unobtainable	160/50	No pulse	120				0.5 L. blood	Prompt response with 2nd dose. Followed with transfusion. Died 14 hours later.
Fracture, skull, jaw, and humerus	Profound shock	Improved	Died	Unobtainable	54/32	No pulse	145	46	35	0	0	B.P. improved markedly from profound shock. Died of skull injury.
Fracture, humerus, both femurs	Severe shock	Good	Good	90/60	110/72	No pulse	96			Saline	0	Saline brought pressure up to 90/50. Albumin gave additional response in 2 doses and B.P. maintained.
Fracture, skull, both tibiae, fibulae, pelvis	Severe shock	Improved	Improved	88/60	115/70	96	100			0.5 L. saline	1 L. saline	Albumin and saline maintained good B.P. in hopeless case long enough for operative treatment.
Compound fracture, both tibiae and fibulae	Severe shock	Fair	Good	80/40	90/45	130	72	40	29	0	1 L. saline	Condition improved and sustained with albumin and saline.
Fracture skull; contusion of brain	Grave	Much improved	B.P. 94/50 before blood	70/40	86/50	144	168			0	5 per cent D in S	Appeared much improved with albumin and saline. Later received 50 per cent D and blood.
Contusion of abdomen	Severe shock	Warm, dry good pulse	Good	?	140/90	Feeble 70	Strong 70	62	48	0	0.5 L. blood post-op.	Striking improvement with albumin alone. At operation, much serous fluid found in abdomen.
Compound fracture femur, multiple lacerations	Mild shock	Good	Satisfactory for 2 hours	90/60	90/60	120	120	36		1 L. D in S	0	Relapsed 2½ hours laters. See below.
Same case 2½ hours later	Moderately severe	Good	Good	60/20	110/60		145		16	0	0	Later transfused because of tachycardia and low hematocrit.
B. HEMORRHAGIC SHOCK												
Bleeding esophageal varices	Shock, cold, wet	Not in shock	B.P. 70/48	80/55	100/76	72	76	8	7.5	0.1 L. saline	Saline continued	Albumin injected into saline tube. B.P. rose in 4 minutes.
Laceration of neck and wrist	Severe shock	Improved	B.P. 115/70	60/?	110/80	120				0	1 L. D in S	Rapid and marked clinical improvement.
Bleeding from splenic artery	Severe; no B.P. for 45 minutes	Fair	Good	Unobtainable	110/60	180	120	45	41	1.25 L. blood 20 cc. eschatin	0.75 L. blood	Patient was not responding to transfusion and exitus considered probable. Albumin response dramatic.
Gastrointestinal hemorrhage	Severe shock	Improved	Improved	70/48	90/40	140 weak	118	19.7	14.7	0	2 hours later 0.52 L. blood	Apparently maintained *status quo* despite continued hemorrhage.
Incomplete abortion	Mild shock	Improved	Improved	80/0	90/60	140	100			0	0.5 L. saline after 45 minutes	Clinical condition showed marked improvement after albumin.
Bleeding duodenal ulcer	Severe shock	Improved	Improved	50/38	112/54	150	100	16.5	14.1	0.5 L. D in S	4 hours later 0.5 L. blood	Immediate response of pulse and blood pressure to albumin.

reading, and blood pressure, and then an attempt was made to evaluate the results in each case, as influenced by the severity and type of injury and by other forms of treatment. A summary of the data in some of the cases of traumatic and hemorrhagic shock is given in Table I.

Eighteen of the cases were in severe or profound shock as a result of very extensive injuries. With one exception, these cases were suffering from fractures, the majority being multiple and 14 of them compounded. Seven had fractures of the skull, 3 others having brain injuries. The reason for the selection of such severe cases was that albumin was usually not requested if the physician treating the case felt that saline alone would suffice.

Twenty-five cases of traumatic shock were treated, among whom the clinical effect was interpreted as good in 17 and fair in 3. The 5 showing no clinical change were all hopeless cases, and in 3 of these, transfusions were of no avail, while in 2 others, the blood pressure and pulse were definitely improved after albumin. Six patients died from the severity of their injuries but showed good response to albumin for a period and would almost certainly have died with any form of therapy; indeed, several were transfused without benefit. The blood pressure recorded in 24 cases showed improvement in 19. Of the remaining 5, 3 continued to bleed, while 2 were *in extremis* at the time of treatment. Hematocrit levels before and after treatment were obtained on 11 and showed prompt hemodilution in all except 2. These cases, although in profound shock, received less than half the usual dose.

Little evidence concerning the duration of the effect of albumin could be gained in these early cases as they were so severely shocked and injured that in only 2 instances was other therapy withheld for more than an hour. In these cases, improvement continued. In 2 cases, the blood pressure rose rapidly and further bleeding occurred, which was difficult to control.

In a few instances, albumin was given prophylactically to patients who, the physician believed, would go into shock. None of these developed shock subsequent to the injection.

Sixteen cases in shock from hemorrhage were treated, with clinical improvement in 13. The response was interpreted as marked or striking in 10. Two who failed to improve had uncontrolled hemorrhage and 1 received less than 12 grams of albumin. Hematocrit readings done on 6 showed a fall in each instance, varying from 5 to 50 per cent.

The operative shock group consisted of 25 cases. With 1 exception, clinical improvement was noted in all who received more than 12 grams. Hematocrit levels were suitable for interpretation in 21 cases and showed hemodilution in all but 1. Three died later because of their disease.

Although transient hemodilution followed the injection of albumin in cases of shock associated with severe infection, the ultimate results were as unsatisfactory as the results obtained by other investigators with blood or plasma (10). In 2 cases of shock from acute abdominal conditions, where there was marked dehydration, the results with concentrated albumin were unsatisfactory until saline solution was administered. Clinical improvement was then noted. That this failure was due to lack of available extravascular fluid was suggested by the fact that although the hematocrit fell somewhat, the serum protein concentration rose more than usual. A fall in both figures occurred following the administration of saline.

Twenty-five cases of burns were treated, 18 of them in the acute phase. Local treatment used in these cases was as follows: tannic acid, 7; triple dye, 3; triethanolamine and sulfadiazine, 3; boric acid ointment and pressure dressings, 1; not reported in others. Several received only concentrated albumin in the first stages, and it was shown that hemoconcentration could be effectively reduced by this treatment. In 1 case, the hemoglobin value of 21 grams per cent was reduced to 16 grams per cent within 1 hour after 25 grams of concentrated albumin. Four hours later the hemoglobin had risen again to 18 grams per cent, and was reduced to 15.4 grams per cent after a second dose. This is probably not ideal treatment, but does demonstrate the effectiveness of concentrated albumin, even in a very severely burned patient (80 per cent of body surface). Many of the patients received albumin as a 5 per cent solu-

tion diluted with physiological saline, and this also was effective in reducing hemoconcentration.

In several cases, the rapid hemoconcentration which follows saline infusion was well illustrated, and the hemoglobin value was brought back to the normal range by the administration of albumin (Figure 5). Since burn patients may require repeated doses over a period of many hours, 1 severely burned patient was treated almost entirely with albumin and saline and glucose solutions for 27 hours in an attempt to determine whether any adverse effects would occur. The burn involved 50 per cent of the body surface, and after debridement, an eschar was formed with tannic acid and silver nitrate. The total dose amounted to 450 grams of albumin (18 passages), and 1 liter of plasma, the equivalent of more than a 2-fold replacement of his total circulating plasma (Figure 5). At the end of 27 hours, his hemoglobin was within normal limits, and he did not go into secondary shock. However, after the first 300 grams (12 passages) had been administered in a period of 18 hours, oozing of blood was observed about the intravenous cannula and it was found that his serum globulin had fallen to an extremely low level (albumin/globulin ratio of 6.7/0.7) and that the prothrombin time was slightly prolonged. Both returned to normal following the use of whole fresh plasma. This is the only case in which were noted the effects of a deficiency of serum globulin following albumin therapy.

A dose of 25 grams was found to be satisfactory in this group of clinical cases, although, in many instances, a second dose was necessary. A dose of 12.5 grams (the equivalent of 250 cc. of citrated plasma) was not sufficient in the majority of cases where this amount was used,

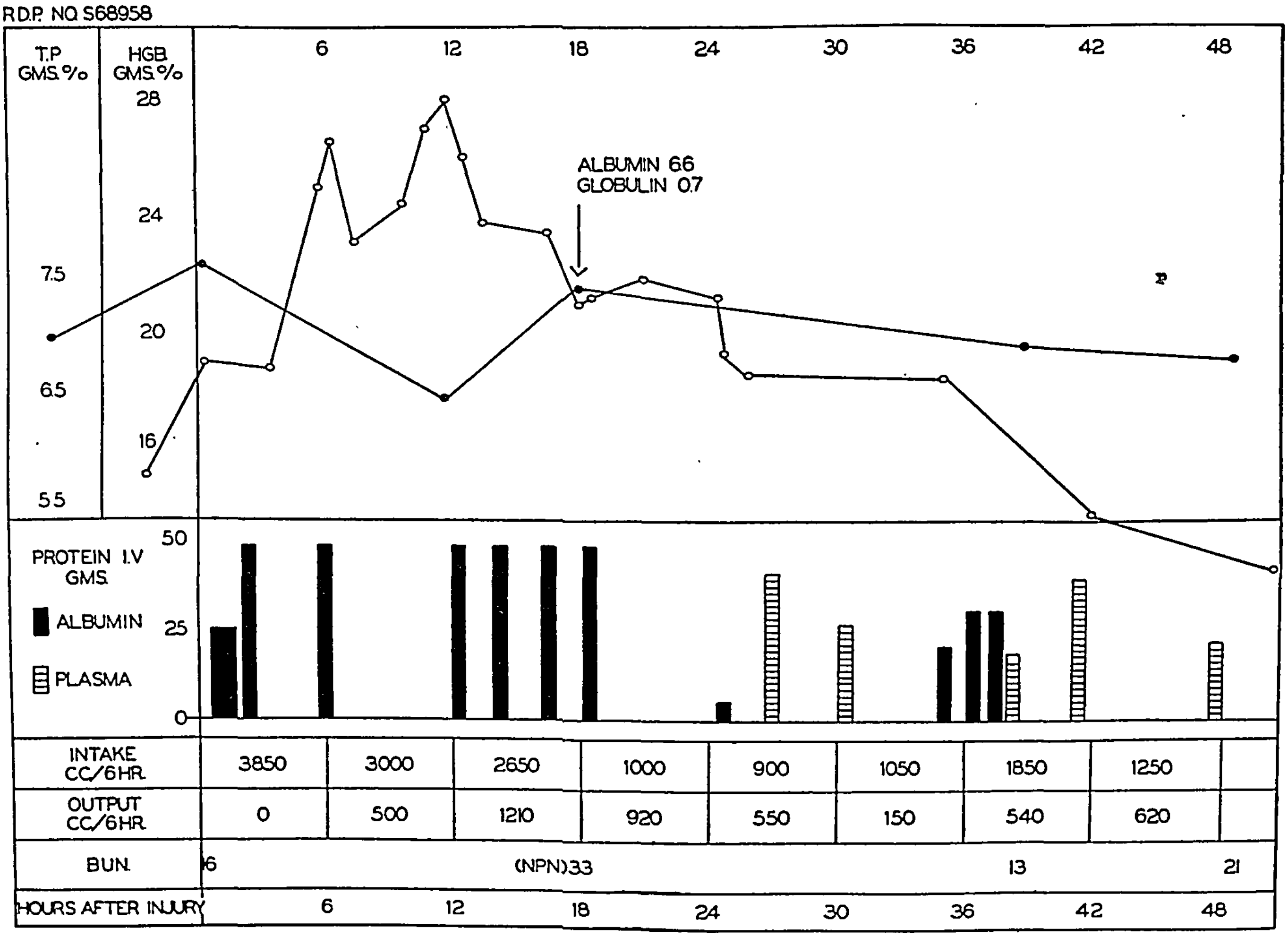

FIG. 5. CLINICAL COURSE OF A SEVERELY BURNED PATIENT (50 PER CENT OF BODY SURFACE) IN THE FIRST 48 HOURS

No fluid was given by mouth during this period. Note rapid hemoconcentration following intensive administration of saline and glucose solutions at 6 and 12 hours and its prompt reduction by albumin injections.

as has been borne out by the decision to increase the size of the Standard Army and Navy dried plasma package to 500 cc. It was our conclusion that the best practice was to infuse 25 grams of albumin rapidly and to repeat this dose about 15 minutes later if a sufficient response had not been obtained. The reason for repeating the injection so promptly was that most of the hemodilution usually occurred within this period (Figures 1 and 4). In burns, there is continued loss of fluid from damaged capillaries, and it is not logical to draw on the extravascular fluid stores for more than emergency treatment. Consequently, we have used albumin diluted to 5 per cent with saline solution (2 packages of albumin per liter of fluid). The amount necessary varied with the extent of the burn and appeared to be comparable to the requirement for plasma.

C. The Standard Army and Navy Package of Serum Albumin

The Standard Army and Navy Package of Serum Albumin was developed in order to provide albumin in compact form, easily available for rapid administration (11, 12). It has been repeatedly demonstrated that this package can be opened and administration started in 1 minute, with less than 10 minutes required for the infusion of its contents by gravity.

The package consists of 100 cc. of a solution containing 25 grams of human serum albumin in 0.3 molar sodium chloride at a pH of approximately 6.8, with merthiolate 1:10,000, or its equivalent, as a preservative. These conditions of solution have been shown in a preceding study (13) to assure adequate thermal stability of albumin. The double-ended glass container with a small vaccine stopper at each end, together with sterile airway needle and complete intravenous equipment, suspension tape, instructions, and questionnaire are sealed in a metal can. The saving of space which concentrated albumin makes possible is best shown by the figures in Table II.

TABLE II

Package size of osmotically equivalent blood substitutes

	Number of units	Plasma volume osmotically equivalent	Size of package	Weight of package
		cu. cm.	*cu. in.*	*lbs.*
Dry plasma				
250 cc. Unit	2	500	480	7.5
500 cc. Unit	1	500	300	5.3
Albumin				
100 cc. Unit	1	500	48*	0.9*

* Taken as one-third of carton containing 3 units.

Discussion

If albumin were dispensed as an isosmotic (5.6 per cent) protein solution, few objections could be raised to its use in the treatment of conditions for which an osmotically active solution is indicated. However, since it is dispensed as a 25 per cent solution, for reasons of military expediency, many may question its use in the treatment of shock. The effectiveness of albumin in augmenting blood volume depends upon its ability to draw fluid from the tissues into the blood stream. When there is sufficient extracellular fluid available, this takes place, as has been demonstrated in the foregoing experimental and clinical studies.

The important question is whether the administration of concentrated albumin to severely dehydrated patients is either ineffective or harmful. The clinical cases shed little light on this, since additional saline solution was administered to the majority of patients and only 5 of the 200 cases were markedly dehydrated. In 2 of these patients only, where there had been vomiting as a result of acute abdominal conditions, was albumin relatively ineffective in producing clinical improvement until additional fluids were given, even though some fall in hematocrit followed its administration. On the basis of the available data, the appraisal committee made the following recommendation, which is stamped on each albumin bottle: "Precaution:—In the presence of dehydration, albumin must be given with or followed by additional fluids." It is not necessary that this fluid be given intravenously if the patient can take water and salt by mouth or retain a proctoclysis.

Subsequently, with crystallized bovine albumin[9] available for animal experimentation,

[9] Prepared at the Armour Laboratories by the method of Cohn and Hughes (14).

data have been obtained which substantiate the wisdom of the committee's recommendation. Fine, Frank, and Seligman (15) have reported that in tourniquet shock in dogs, which in their hands was almost uniformly fatal if untreated, concentrated albumin was ineffective. However, if saline were given by stomach tube, 25 per cent albumin was as effective as 5 per cent albumin or plasma. Their protocols do not show that concentrated albumin was harmful *per se*, but in the absence of sufficient extravascular fluid, it failed to produce an adequate increase in plasma volume. Mahoney, Kingsley, and Howland (16) have shown that in the treatment of severe shock due to intestinal trauma in dogs, dilute and normal plasma were more effective than concentrated plasma. Twenty-five per cent albumin did more than concentrated plasma to restore the circulation, but the animals did not survive (17). Dunphy and Gibson (18) compared the effect of concentrated and dilute albumin solutions with homologous plasma in the treatment of severe burns in dogs. Concentrated albumin was effective in sustaining the circulation, but microscopic examination seemed to indicate slightly greater tissue damage in animals receiving the concentrated solution as compared to dogs receiving plasma or dilute albumin. We bled 2 splenectomized dogs, which had been dehydrated by withholding all water for 72 hours, until the blood pressure fell to shock levels, and the animals were stuporous and cold. Large doses of concentrated crystallized bovine albumin were then injected, and the animals responded with a rise in blood pressure, hemodilution, and marked clinical improvement, so that they were able to get up and walk about the room, somewhat unsteadily. The serum protein concentration rose from 6.5 to 8 grams per cent. Four hours after albumin the dogs were offered water and saline solution. They drank both and then became normal in behavior, a fall in serum protein and hematocrit showing that further hemodilution had occurred.

Thus, the experimental data indicate that the effectiveness of concentrated albumin in the treatment of blood and plasma loss may be somewhat limited by the extent to which the patient is dehydrated, although this factor was not important in actual cases of shock due to trauma or hemorrhage. There is little evidence that albumin is harmful even in the presence of severe dehydration, but the deficiencies of fluid and electrolytes, characteristic of this condition, should be remedied as soon as possible if a maximum therapeutic effect is to be obtained.

A second objection, frequently raised to the use of albumin, is that since it lacks the globulins of the plasma, its administration in large doses may lead to a deficiency of those globulins concerned with blood coagulation and immunity. Earlier studies in dogs and humans receiving bovine albumin showed that there was a considerable margin of safety before a diminution of the antibacterial or phagocytic powers of the blood could be detected (19). In our group of patients, there was nothing to suggest a lack of immune bodies. Relative globulin deficiency, as indicated by a low serum globulin concentration was observed only twice, and in only 1 patient did oozing of blood occur which could be correlated with a prolonged prothrombin time. A comparison of the relative ability of albumin and aged liquid plasma, which contains no functionally active but only denatured globulins, to promote the regeneration of prothrombin and complement should be both interesting and important. In severe burns, plasma rather than albumin is obviously the therapeutic agent of choice, but we have demonstrated in 1 patient at least that the osmotic equivalent of nearly twice the calculated plasma volume can be replaced with albumin and saline solution before serious signs of globulin deficiency appear.

It should be emphasized that neither albumin nor plasma supply red cells, with their capacity for transporting oxygen. Thus, the continued use of protein solutions, to replace losses of both plasma and blood in patients with war injuries, will inevitably result in the development of anemia, unless red cells are given to make up this deficit.

Since a small volume of albumin injected intravenously induces a considerably greater increase in the volume of the circulation, some caution should be used in its administration to patients with a low cardiac reserve. We have observed a few cases of shock in whom bleeding had not been controlled before treatment, who lost

considerably more blood when the blood pressure rose rapidly after albumin injection. This is a principle of hemodynamics which applies to any effective form of therapy.

Summary of Part I

1. Concentrated human serum albumin increases blood volume rapidly by drawing extravascular fluid into the circulation.
2. The hemodilution which follows albumin injection is well sustained in patients with previously depleted blood volumes, but is transient in those with normal blood volumes.
3. As would be expected from *in vitro* measurements, approximately 18 cc. of fluid is added to the blood stream for each gram of albumin injected after hemorrhage, with wide variation in individual instances. Thus, the standard package of 25 grams of albumin in 100 cc. of diluent is equivalent in its osmotic effect to 500 cc. of citrated plasma.
4. Prompt hemodilution and clinical improvement have been shown to follow the injection of concentrated albumin in a group of cases of shock and burns.
5. Clinical and experimental data indicate that concentrated albumin is not harmful in cases of shock with severe dehydration, but is more effective if water and salt are also administered by any available route.
6. Clinical effects of a deficiency of the serum globulins have been noted in only 1 case—a severely burned patient, who had received 300 grams (12 passages) of albumin in 18 hours.
7. The Standard Army and Navy Package of Normal Serum Albumin (Human) concentrated, provides a stable blood derivative in compact form, instantly available for rapid administration, which has been developed to meet the needs of highly mobile military units.

PART. II. SAFETY OF ALBUMIN

As commercial production of albumin for the armed forces began, a rigid system of control was considered necessary. Therefore, after each lot of albumin had undergone physicochemical as well as the usual sterility, animal safety, and rabbit pyrogen tests, samples were used clinically before it was released.

A. Methods of study

Since more than 1900 such intravenous injections of albumin have been given in the past 20 months, it is evident that it was not feasible to test each sample on a separate subject. Accordingly it was decided to give multiple injections whenever possible to patients with hypoproteinemia, who might be expected to benefit from albumin therapy. In addition to a diminished serum protein level, the cases had to have a relatively normal temperature. Those with cardiac disease or allergy were excluded. Patients selected according to the above criteria were given the test lots of albumin by gravity, using the intravenous kit included in the package. The temperature and pulse were recorded before injection and each hour thereafter for 3 to 4 hours, the patients being carefully observed during this period.[10] The albumin was administered as the standard 25 per cent solution in 0.15 or 0.3 molar sodium chloride, buffered with sodium bicarbonate to a pH of from 6.6 to 7.0, and containing 1:10,000 merthiolate, or its equivalent, as a preservative. A dose of 20 to 25 grams was given at each injection.

B. Results

1. Amounts given

From April 1942 (when the first standard albumin prepared for the armed forces was tested) to December 1943, 1915 injections of albumin were given to 600 patients. This included 250 injections of material heated for varying periods at different temperatures before administration. Forty-four patients received more than 10 injections. Five patients received over 800 grams each, 3 of these receiving over 1000 grams. The 3 longest periods of treatment extended over 69, 342, and 570 days. Six patients received an average of 25 or more grams of albumin daily for from 5 to 20 days.

2. Reactions

In our hands, properly processed albumin has been administered routinely without reaction. However, in the use of albumin submitted for approval, particularly in the early days of commercial production, we encountered a certain

[10] We are indebted to the members of the visiting, house, and nursing staffs of the following hospitals for their cooperation: Beth Israel, Children's and Infants', Boston City, Boston Floating, Massachusetts General, Massachusetts Memorial, and Peter Bent Brigham Hospitals, Boston, Mass.; U. S. Naval Hospitals in Chelsea, Mass. and Bethesda, Md.; Doctor's, Gallinger Memorial, Garfield, Providence, and Sibley Hospitals, Washington, D. C.

number of pyrogenic reactions. Such lots were rejected until the albumin had been reprocessed and repackaged in a satisfactory state. Data are not available on the incidence of reactions in routine use of the lots which have been released, but since no preparation which gave reactions on test has been accepted, it should be extremely low.

In all, seventy-seven pyrogenic reactions occurred (in the course of these studies) in 56 patients given 39 lots. Fifty-five reactions consisted of chills and fever, while in 22, fever was the only manifestation. The average recorded temperature rise was about 2° F. The interval before onset of chills was usually under 1 hour with an average duration of about 30 minutes. No anaphylactoid symptoms were observed, following single or repeated injections of standard lots.

Cardiovascular symptoms due to rapid increase in the volume of the circulation appeared in some, such as increased pulse and blood pressure, but no pulmonary edema was seen.[11] Severe pyrogenic reactions in 3 patients were followed by hematemesis from esophageal varices. One patient with nephrosis died in convulsions the day after a severe pyrogenic reaction, which was presumably due to bacterial contamination of a single bottle, since intensive study of the rest of this lot showed that it was satisfactory. Three patients died from pulmonary embolism within 24 hours of albumin injection.

Since albumin was developed for use in places where refrigeration is not available and the weather apt to be hot, it was important to demonstrate that material which had been exposed to a high temperature did not produce reactions. Actually, the incidence of pyrogenic reactions was reduced by exposure of albumin to heat. Some heated samples showed visible changes, consisting of a darker color, slight turbidity, and fine floccules. Many of these have been administered without difficulty, but to assure safety, a fine wire mesh filter has been incorporated in the intravenous equipment of the standard package. In the group of 250 patients given heated albumin, no anaphylactoid reactions occurred. One hundred and thirty-three of these patients (Table III), as well as 72 who received injections of unheated albumin, were followed for from 3 weeks to one year, and no delayed reactions of any kind were observed. No cases of homologous serum jaundice have been discovered (20).

TABLE III

Injections of heated albumin

Temp. at which heated	Days heated	No. injections	No. followed	Reactions	
				Early	Late
°C.					
37	77 to 134	3	2	0	0
45	31 to 60	2	0	0	
50	1 to 10	15	0	0	
50	12	218	131	6*	0
50	14 to 33	6	0	0	
50	100 to 117	6	0	0	
	Totals	250	133	6*	0

* Pyrogenic reactions in 2 patients with each of 3 unsatisfactory lots.

3. *Pathologic studies*

While the evaluation of human serum albumin chiefly involved clinical and physiologic technics, it seemed important to determine whether or not pathologic changes were produced by the material. Since the use of experimental animals would have introduced the complicating factors of species differences, it was necessary to depend upon human necropsy material for pathologic data. It was essential to attempt the separation of any pathologic changes due to the human serum albumin itself from those due to the preservative (merthiolate) and those due to the disease from which the patient was suffering. While minute histologic alterations resulting from the administration of the albumin might well be masked in such material, tissue changes of importance would be detected.

Several hospitals[12] contributed material for

[11] One patient developed pulmonary edema following repeated albumin injections in the early days of clinical testing, before the methods for the preparation of albumin had become standardized.

[12] We wish to express our gratitude to those hospitals which submitted material to us: Peter Bent Brigham, Massachusetts General, Children's, and Boston City Hospitals, Boston; Johns Hopkins Hospital, Baltimore; and New York and Presbyterian Hospitals, New York.

this study. There was necropsy material from 16 patients who received from 13 to 813 grams of human serum albumin. Included in the series were patients dying within a few hours after a single injection, as well as those receiving large quantities in repeated injections over several months.

The results of the examination of tissues from these 16 patients may be summarized briefly. In no organ was there evidence of histologic change which could be correlated with the administration of albumin. Particular search was made for "storage disease," which was not found. Pathologic storage of this material would not be expected in view of the fact that it is native to the human organism and is an integral part of the blood plasma. However, these studies offer objective proof that pathologic accumulation does not take place, even when as much as 813 grams are administered. There was no evidence of periarteritis nodosa in any instance. No renal glomerular damage was encountered which could not be explained by the disease of which the patient died. It was in the evaluation of minute changes in renal morphology that the shortcomings of routine necropsy material were most evident.

One patient (C. B.) with intestinal obstruction and bronchiogenic carcinoma, receiving 650 grams of human serum albumin in 6 days, deserves special comment. The albumin was administered in 25 per cent solution containing 1:10,000 merthiolate, a total of approximately 0.13 gram of mercury. In addition, the patient received 2 doses of salyrgan, containing a total of 0.08 gram of mercury, the last on the day of death. There was considerable swelling, degeneration, and desquamation of the cells of the convoluted tubules of the kidney. In another patient receiving 813 grams of albumin over a period of 2 months, there was less evidence of damage to the renal tubules. The evaluation of these changes was particularly difficult because of the numerous pathologic alterations due to the diseases for which the albumin was given. It seems likely that they were due to the mercury rather than to the human serum albumin *per se*. However, the changes were very minor and could not be interpreted as affecting the clinical course of the patient.

Discussion

The occurrence of pyrogenic reactions in a few lots which had passed the rabbit pyrogen test required by the National Institute of Health justifies the procedure of clinical testing, and since these lots were rejected, the reaction rate with albumin released for distribution should be very low. Most pyrogenic reactions have been mild but have had serious consequences in a few instances. Hematemesis from esophageal varices after a severe chill and pulmonary embolism following a sudden increase in blood volume are not unlike accidents which have been observed after blood or plasma transfusions in any large group of patients with serious chronic illnesses.

The fact that a few patients have received total doses of over 1000 grams, that others have tolerated repeated injections at varying intervals, and that heated albumin has been injected repeatedly without the occurrence of anaphylactoid symptoms, indicates that standard preparations of concentrated human albumin are not antigenic in man, nor do they become so on heating at 50° C.

Summary of Part II

1. In clinical trials of individual preparations of concentrated human serum albumin, 1915 injections were given to 600 patients.

2. Pyrogenic reactions occurred with unsatisfactorily processed material, which was rejected. The reaction rate from albumin lots released for distribution under the rigid system of control used should be extremely low.

3. In 7 of a group of 600 patients, most of whom were suffering from serious chronic illnesses, untoward incidents occurred within a short time after albumin injection. These are discussed in the text.

4. Repeated injections of normal or heated (50° C.) albumin failed to produce any other type of immediate or delayed reaction.

5. Five patients received a total of more than 800 grams of albumin each, 6 patients received 25 or more grams per day for from 5 to 20 days, and 44 patients received more than 10 injections without harmful effects.

6. Pathologic studies of necropsy material from patients who had received albumin failed

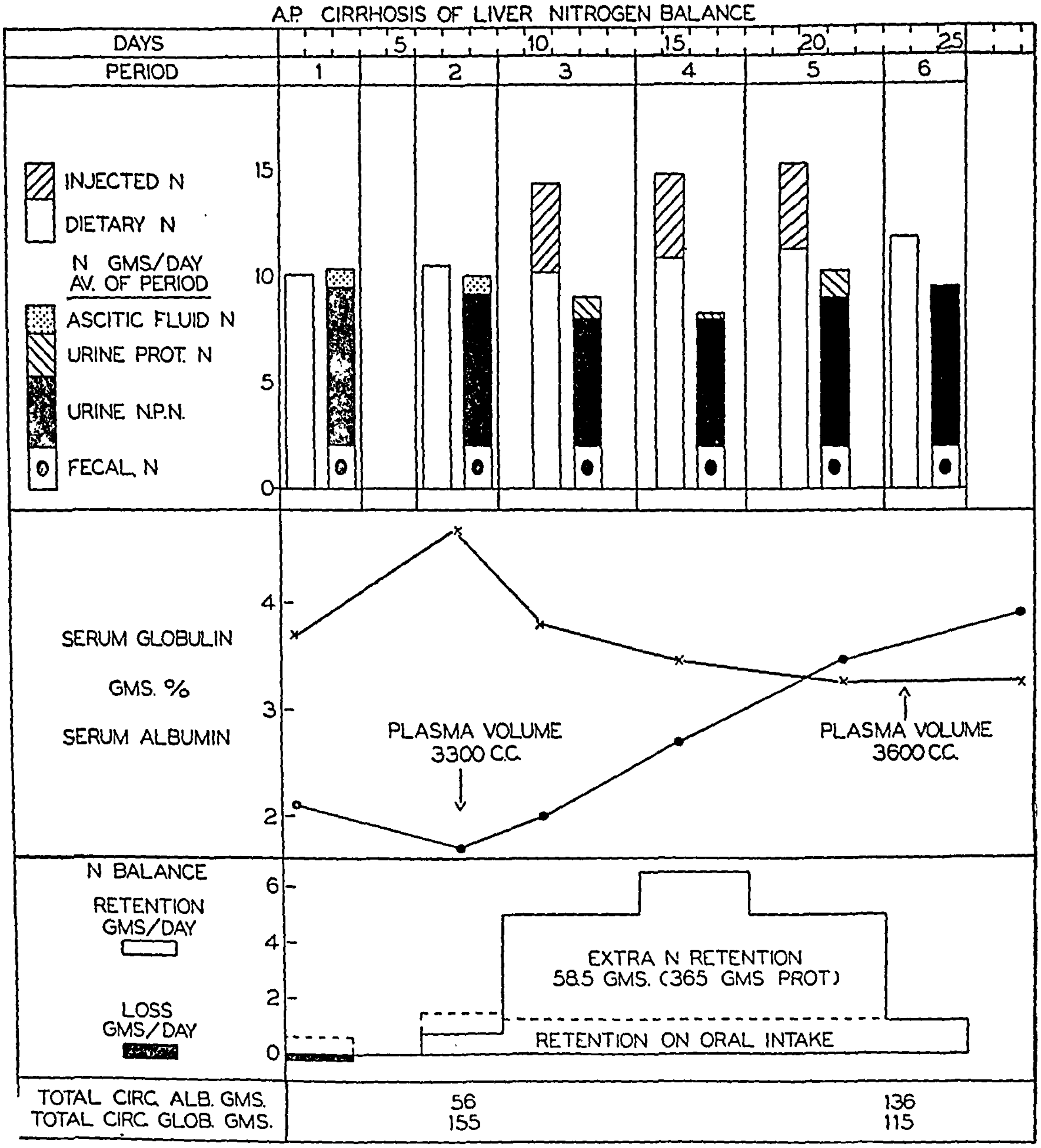

FIG. 6. NITROGEN BALANCE STUDIES IN A PATIENT (A. P.) WITH CIRRHOSIS OF THE LIVER WHO RECEIVED 350 GRAMS OF ALBUMIN IN PERIODS 3, 4, AND 5

and II, interpretation was complicated by variations in the daily protein intake. In patient I, with the nephrotic syndrome, increased proteinuria accounted for a portion of the injected albumin. In patient III, it will be noted that the extra nitrogen retained during the period of albumin injection corresponded very closely with the extra nitrogen injected in the form of albumin. Although the full 350 grams of protein injected were retained, only about 80 grams or one-fourth of it could be accounted for by the increase in total circulating albumin.

Thus, albumin is presumably stored in hypoproteinemic patients, since there is no appreciable increase in nitrogen excretion, and it do[es] not appear in the urine unless there is a tendenc[y] to proteinuria. This is consistent with the be[-]havior of native plasma protein, and sugges[ts] that the albumin molecule has not been sig[-]nificantly altered by the process of fractionatio[n].

2. Results in the treatment of hypoproteinemia

Because of the large amounts of protein neces[-]sary to achieve a therapeutic result in hypopro[-]teinemia, only a few patients can be said t[o] have received adequate doses. These patient[s] may be divided into several groups, accordin[g] to the major cause of hypoproteinemia; (*a*) thos[e]

with an inadequate protein intake; (*b*) those with altered ability to synthesize serum albumin; (*c*) those with excessive loss of protein from the body; and (*d*) a group of miscellaneous cases.

(*a*) *Hypoproteinemia due to inadequate protein intake.*

Cases

(*1*) E. M., an emaciated woman in her seventies, entered the hospital because of weakness, dyspnea, and ankle edema. She had been living on a meager allowance, very little of which was spent on food. On admission, attention was focussed on her heart because of paroxysmal auricular fibrillation, râles, pleural effusion, and ankle edema. Despite digitalis and rest in bed, her general condition improved little in the first 3 weeks, and, on a ward diet with vitamin supplements, the serum protein value fell from 5.6 to 5.1 grams per cent. At this point, without change in her diet, treatment with albumin was begun in doses of 20 to 25 grams nearly every day. The results were striking; strength, alertness, and appetite improved. No cardiac embarrassment occurred. Her edema, which had partially subsided before the institution of albumin treatment, disappeared completely, and she left the hospital markedly improved 66 days after admission. The total dose of albumin administered in 40 days to this woman weighing only 84 lbs. was 660 grams. The rise in serum albumin, shown in Table VI, accounted for only a small portion of that administered.

TABLE VI

Changes in serum proteins of patient E. M. with chronic malnutrition

Date	Albumin injected	Serum albumin	Serum globulin	Total protein
	grams	*grams per cent*		
June 15, 1943	0.0			5.6
July 4, 1943	0.0	2.8	2.3	5.1
July 16, 1943	180.0	3.9	3.0	6.9
August 11, 1943	600.0	5.0	3.0	8.0

(*2*) W. S. was a 71-year-old man who developed symptoms of intestinal obstruction, proved at necropsy to be due to stricture of the ileum. His serum protein level was extremely low (albumin 1.1 per cent, globulin 2.3 per cent), and therefore, after Wangensteen suction was instituted, he was given daily injections of albumin for 11 days. Because of difficulty with veins from massive edema, this was administered intrasternally through a needle which was left in place between injections. The albumin flowed in readily for the first 3 days, but ran more slowly thereafter, and none could be administered by this route after a week. He took only a small amount of nourishment by mouth and received daily infusions of glucose and albumin. After 320 grams had been given over an 11-day period, there was some improvement of his edema and his serum proteins had risen (albumin 2.0 per cent, globulin 2.0 per cent), but he failed to gain sufficient strength for operative treatment and died.

(*3*) C. B., mentioned in Part II, had severe hypoproteinemia following an operation for intestinal obstruction. Large amounts of albumin were available and he was given as much as 125 grams per day, a total of 650 grams in 6 days. This raised the serum albumin level from 2.4 grams per cent to a final value of 5.2, with a serum globulin level of 0.7 per cent. There was marked reduction in edema and clinical improvement at first, but signs of pulmonary congestion subsequently appeared and the patient died in pulmonary edema following an injection of salyrgan the day after his last dose of albumin. The postmortem findings included generalized edema, carcinoma of the lung, and minor changes in the renal tubules which were attributed to mercury.

From these 3 patients, it was concluded that the serum albumin level can be raised by the administration of large doses of albumin to severely depleted patients. While the second patient did not receive sufficient albumin to relieve his deficiency, it seems probable in the third case that the pulmonary congestion and edema were at least partly due to excessive doses of albumin. Thus, two limiting factors to the speed with which the serum albumin level can be brought back to normal appear to be (*1*) the capacity of the circulation to adjust to rapid increases in plasma volume, and (*2*) the rate at which the tissues can metabolize or store excess albumin.

(*b*) *Patients with altered synthesis of serum albumin.* Cases of portal cirrhosis with low serum albumin levels were treated because they are frequently unable to maintain an adequate level of serum albumin, even on a high protein diet (25). Most of the patients in this group had ascites, and all had had repeated paracenteses. Although it was felt that the ascites was due to at least two factors, increased venous pressure in the portal system, and low total colloid osmotic pressure of the blood, it was hoped that if the serum albumin level could be raised sufficiently, the rate of formation of ascitic fluid might be diminished.

Cases

(*1*) A. P. was a 65-year-old woman whose condition had been diagnosed as portal cirrhosis, 14 months previously. At that time, 3 paracenteses were required in 2 months. Abdominal fluid then accumulated slowly until 1 year later, when 25 liters were removed in 5 paracenteses, the last 2 of which were performed after admission to the

hospital. At this time, she was in a good state of nutrition and complained only of the discomfort from her protuberant abdomen. Her spleen was not enlarged, and no esophageal varices were demonstrated.

She was placed on a 65-gram protein diet and 25 grams of albumin were given daily for 14 days, during which time she lost a slight amount of weight. Her serum albumin level increased from 1.7 to 3.9 grams per cent during this period, whereas her serum globulin decreased from 4.7 to 3.8 grams per cent. Nitrogen balance studies (Figure 6 and Table V) showed complete retention of the injected albumin. Electrophoretic patterns of her serum before and after treatment (Figure 7) clearly indicated the increase

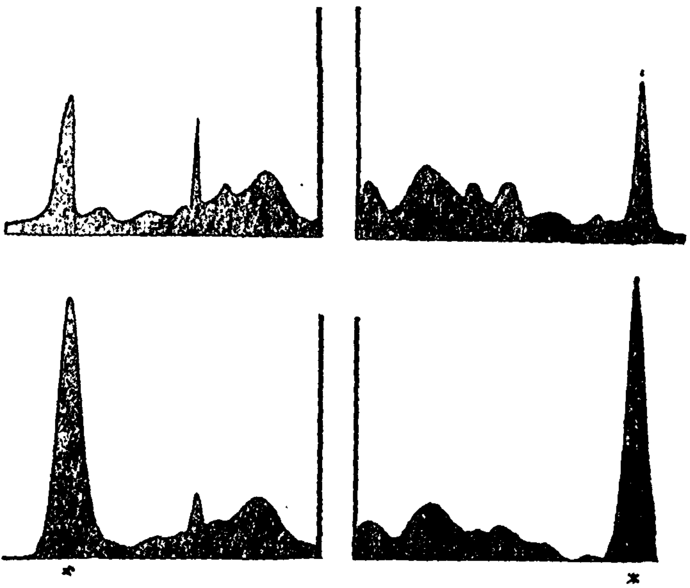

FIG. 7. ELECTROPHORETIC SCHLIEREN DIAGRAMS OF THE SERUM OF PATIENT A. P. WITH CIRRHOSIS OF THE LIVER BEFORE (UPPER) AND AFTER (LOWER) TREATMENT WITH 350 GRAMS OF ALBUMIN

Note increase in albumin fraction (*).

in albumin. A bromsulfalein test on admission showed 60 per cent retention in 30 minutes, and this was recorded as 30 per cent following albumin therapy.

She was then followed at intervals in the clinic, her weight remaining essentially constant during the next 6 months despite the fact that the serum albumin had fallen, during the 2 months after discharge, to 2.5 grams per cent. It remained at this level during the next 4 months without increase in abdominal fluid. When seen 10 months after discharge, her serum albumin had fallen to 2.1 grams per cent and during the preceding months, she had noted a distressing increase in ascitic fluid. At this time, it was felt she was entering the terminal stages of hepatic insufficiency, and she required 5 more paracenteses in the next 2 months.

It was concluded from this case that the serum albumin level in cirrhosis could be elevated if sufficiently large doses were given, but that only a portion of the injected protein would be retained in the circulation. The increase in serum albumin was lost in a period of 2 months, and the interval between series of paracenteses did not seem appreciably affected.

(2) M. C., another patient with portal cirrhosis (Figure 8) entered the hospital, bed-ridden and emaciated. His abdomen was very distended, and a large spleen and esophageal varices were found. A series of 3 paracenteses removed about 20 liters of fluid. At this time, the serum albumin was 1.6 grams per cent and the serum globulin 6.2. For 10 days, the patient was given daily doses of 25 grams of albumin, and at the end of this period, 3.8 liters of fluid were removed from his abdomen. The serum albumin level had risen to 3.5 grams per cent at this time and the globulin had fallen to 4.1. During the next 7 weeks, he received 34 more injections of albumin with but slight further change in his serum proteins and a steady decline of his hemoglobin concentration. His weight increased slowly, levelling off when the abdomen became tightly distended. Fourteen liters of fluid were removed in the 2 weeks following treatment, and at the end of this time, the serum albumin level had already decreased to 2.3 grams per cent. Despite this, he did not require removal of fluid for 6 weeks, a period quite comparable to that while in the hospital. The one striking change noted in this patient was the improvement in general condition during treatment. Although bed-ridden, emaciated, and somewhat delirious on admission, he became stronger, his color and appearance improved, and by the time of discharge he was up and about. Three months later, when seen for the last time, his general condition again approached that at the time of admission, and death occurred a few weeks later.

This patient received very large amounts of albumin (950 grams) during a period of 8 weeks, but in spite of the fact that the serum albumin rose quite satisfactorily within the first 2 weeks, abdominal fluid continued to accumulate at nearly the same rate, and when treatment was omitted the albumin level in the blood dropped soon afterwards. Only the improvement in his general state of nutrition seemed noteworthy.

Four other cases of portal cirrhosis, all of them advanced, were treated and confirmed the previous findings. The serum albumin level was raised to some extent in each case, usually with a slight fall in serum globulin, and in several, there was a distinct improvement in nutrition. The evidence is not adequate to determine whether or not albumin therapy affected the rate of ascitic fluid formation. It is true that in several patients fluid did not reaccumulate for several months after treatment, but these patients had exhibited similar remissions of ascites,

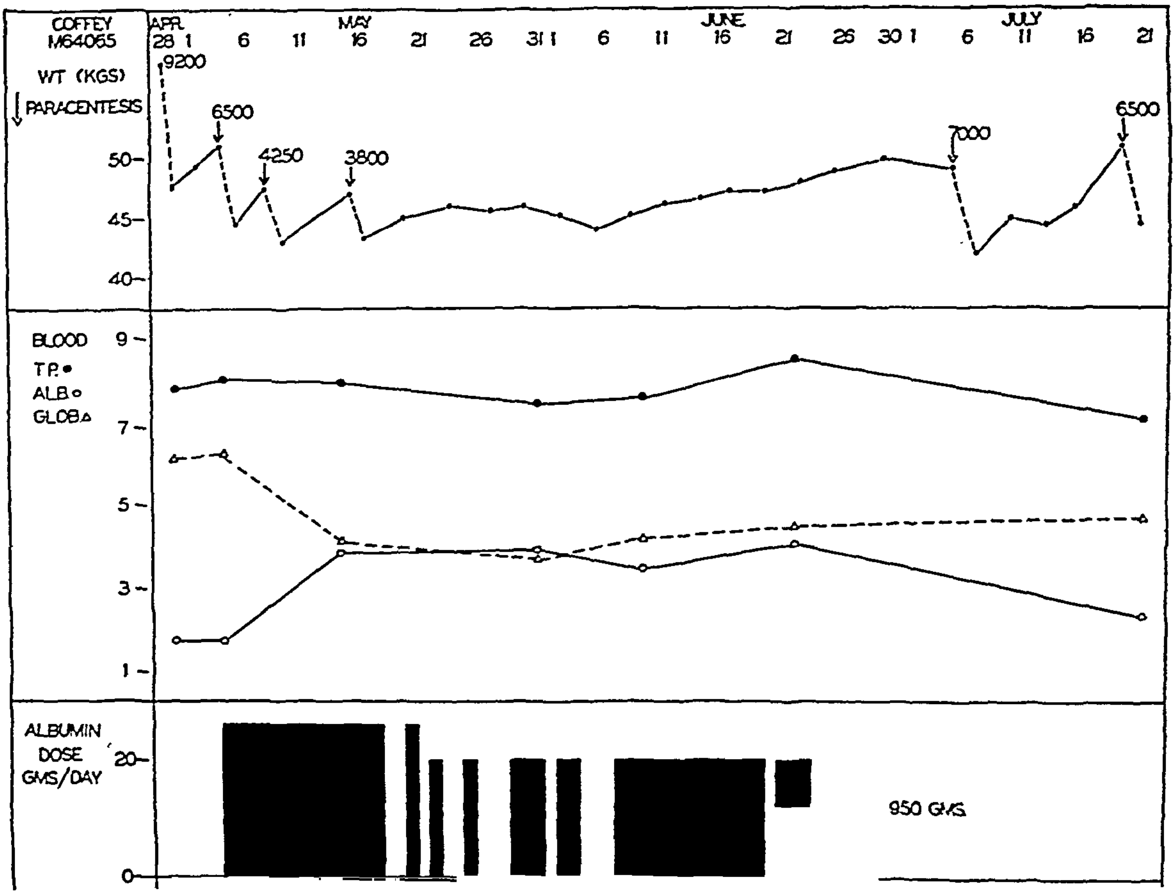

FIG. 8. CLINICAL COURSE OF PATIENT M. C. WITH CIRRHOSIS OF THE LIVER WHO RECEIVED 950 GRAMS OF ALBUMIN IN 8 WEEKS

Note sharp rise in serum albumin level at first and rapid fall after treatment was stopped.

previously. Our findings suggest that although ascites in these cases is usually associated with a diminished concentration of serum albumin, reduction in the colloid osmotic pressure of the blood is not the only factor in its production.

(c) *Patients with excessive protein loss.* Our third group of cases with hypoalbuminemia includes patients with proteinuria of sufficient degree to have depleted the plasma proteins. A few such cases have been treated, most of whom were in the nephrotic stage of chronic glomerulonephritis, while 2 children were classified as cases of true nephrosis, and another patient as a case of amyloid nephrosis. They were all similar in that they had low levels of serum albumin, they were losing large amounts of protein in the urine, and they were edematous.

Nephrotic edema has come to be regarded as the result of lowered colloid osmotic pressure of the blood, due to loss of plasma proteins, particularly albumin, through the kidney. The logical assumption from this concept has been that if the total colloid osmotic pressure of the blood could be raised *sufficiently, diuresis with loss of* edema should occur.

Blood plasma and gum acacia have been widely used in the treatment of such cases with some success. *A high protein diet has proved of* value in aiding the synthesis of serum proteins and protecting the stores of the individual. It was felt that the injection of *normal serum* albumin *might help to raise the level of this* substance in the blood. It should be desirable to bring the serum albumin level to normal limits fairly rapidly, but due to its high osmotic activity, the *amount of albumin which can be* injected in a short period is definitely limited, since if given in *excessive doses,* the circulation

may become overloaded. For this reason, but mainly because the albumin testing program usually would not permit the administration of more than 1 bottle per day to a patient, we have restricted ourselves to doses of 25 grams per day in most instances. The albumin was given as a 25 per cent solution in all cases. This solution contained 0.85 per cent sodium chloride in cases 1 and 2, and 1.7 per cent sodium chloride in cases 3, 4, and 5.

Cases

(*1*) J. C., a girl aged 2, with chronic glomerulonephritis, was very edematous, her abdomen distended with fluid, and her eyes closed by swelling. She had previously been treated with blood, plasma, and urea. Three hundred grams of albumin were given in 10 to 25 gram doses, daily for 2 weeks. Her edema was reduced in amount and her weight decreased from 18.6 to 16.0 kilograms. Although her output was increased, the amount could not be measured. One determination of the urinary protein concentration, several weeks before treatment, was 5 Esbach units, and during albumin therapy it rose to 10 units, but the total excretion is not known. Her serum albumin and globulin levels were 1.5 and 1.6 grams per cent, respectively, before treatment, and these changed very slightly to 1.8 and 1.2 grams per cent. When treatment was stopped because of lack of material, her weight promptly rose.

From this it was realized that very large doses of albumin would be necessary in these patients, as this child, weighing less than 16 kilograms, received 300 grams of albumin with only a slight effect on the serum albumin level.

(*2*) B. B., a patient with chronic glomerulonephritis in the nephrotic stage, was a 27-year-old male, who had had 2 previous admissions for the same disorder. On the first, he had been treated with plasma and acacia, and on the second, with casein hydrolysate. Each time there was a complete diuresis within a few weeks and he became asymptomatic for a period of months. The direct relation of treatment to diuresis could not be established. On this admission, his edema was much more persistent and he was in the hospital for more than 8 months before diuresis occurred. He was first given plasma and casein hydrolysate (1020 grams), but his weight continued to increase. In August 1942, his serum albumin level had fallen to 0.7 gram per cent, and on 2 consecutive days, his proteinuria amounted to 13 grams per day. During the next 22 days, he received 150 grams of plasma protein and 175 grams of albumin as shown in Figure 9. His serum albumin during this period rose to 1.0 per cent, with a somewhat greater

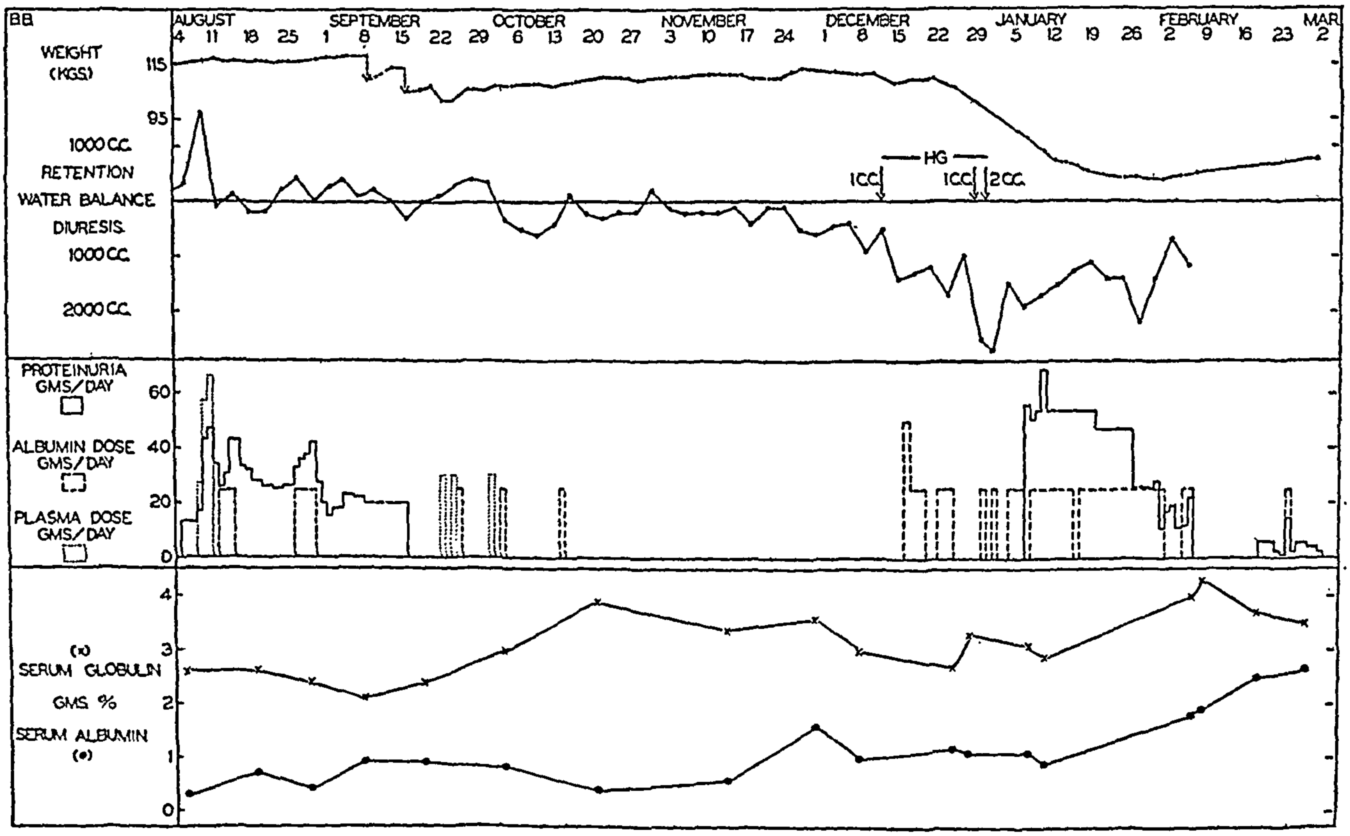

FIG. 9. CLINICAL COURSE OF PATIENT B. B. IN NEPHROTIC STAGE OF CHRONIC GLOMERULONEPHRITIS
Note increased proteinuria coinciding with injections of albumin or plasma.

increase in globulin, but the former soon fell to its previous low level. During the next 2½ months, his weight increased steadily (aside from the effects of 2 paracenteses), and he continued to retain water. From daily determinations of his protein excretion, it was found that immediately following each injection of protein, there was a marked increase in protein loss, so that within a few days, this appeared to account for the major portion of what had been given, although his nitrogen balance (Table V; I) had become somewhat more positive.

In late November, the patient's weight reached a maximum and started to decline. This was immediately preceded by a change to a consistent and increasingly negative water balance. When diuresis was well established, 300 grams of albumin were administered over a period of 12 days, but the serum albumin level did not change significantly. The urinary protein loss was again measured and found to be over 50 grams per day, a level which it maintained for the next 2 weeks, during which almost daily injections of 25 grams were given. When his weight had fallen and he became edema-free, the amount of protein excreted fell rapidly, over a period of several days. With this decrease, it became evident that the injected albumin was now being retained, and this was soon reflected by a sharp rise in serum albumin. The patient's diuresis was then complete. Two weeks later, his urine was found to contain only 3 to 5 grams of protein per day, but following one albumin injection of 25 grams, he appears to have excreted over half of it within 24 hours.

Our conclusion from this case was that the large doses of albumin injected were followed by a corresponding increase in protein excretion, and only when diuresis was nearly complete, and the degree of proteinuria had diminished, could the injected albumin be accounted for in the circulation.

(*3*) D. F., an 8-year-old boy, was admitted because of a puffy face and swollen scrotum. The urine contained large amounts of albumin and a very rare red cell. The edema was of a week's duration and had previously been present on only 2 occasions, lasting one day each. He was placed on a high protein, low salt diet, with restricted fluids, and diuresis commenced immediately, with a fall in weight for 3 days (Figure 10). It then stopped, however, and the weight was regained in a similar period. His serum albumin and globulin were 0.5 and 3.5 grams per cent, respectively, at this time. Albumin was then given intravenously for 30 days in doses of 20 to 25 grams per day, and his daily protein excretion increased markedly. Diuresis set in on the day albumin was started and his weight began to decrease. His serum albumin level for the first 10 days showed little change, despite the 225 grams of albumin administered. As the diuresis progressed, however, the proteinuria diminished sharply until it was evident that the injected albumin was being retained. Coincident with this decrease in protein excretion, his serum albumin level rose rapidly to normal. When seen 6 months later, he was found to have been free of symptoms, his urine contained no protein, and his serum protein value was 7.2 grams per cent, with an albumin level of 4.5 per cent.

(*4*) D. Fa.,[11] a girl of 21 months, with nephrosis, who had been in the hospital for several months without improvement, received 500 grams of albumin in a period of 20 days, an average daily dose of 25 grams. With treatment, there was a marked diuresis, disappearance of edema, and fall in weight from 12.6 to 8.4 kilograms, while her general condition improved markedly. Serum albumin and globulin values before treatment were 1.3 and 5.1 grams per cent, respectively, and afterwards 4.8 and 1.1 grams per cent. She was still improved clinically when seen 2 months later, but proteinuria was still present.

(*5*) E. H., a 27-year-old male, with chronic glomerulonephritis in the nephrotic stage, failed to improve despite the administration of a total of 565 grams in 15 to 25 gram doses, almost daily for 30 days. His weight increased steadily with continued accumulation of edema. His serum albumin level remained almost constant at 1.7 grams per cent, and marked proteinuria was present throughout his course. It was felt that he was doing so poorly that albumin should be discontinued.

One patient, B. F., suffering from generalized amyloid disease was given 250 grams of albumin in 10 days with an increase in serum albumin of only 0.5 gram per cent and some diuresis. Two other children in the nephrotic stage of chronic glomerular nephritis were treated. One, E. W., a 5-year-old child, received almost daily doses of 12 grams for 5 weeks without visible evidence of improvement, her serum albumin increasing from 0.9 to 1.2 grams per cent. The other, M. Ca., received 50 grams in 4 doses without effect except for an increase in proteinuria from 3.9 to 10 Esbach units, without change in urine volume. Treatment was stopped because of the difficulty in finding suitable veins. One adult patient, W. M., received over 1000 grams in a period of 6 months. During this time, his serum albumin rose only from 2.3 to 2.7 grams per cent, whereas his globulin rose from 1.5 to 2.8 grams per cent, and remission of edema occurred.

(*d*) *Miscellaneous cases.* Albumin has been given to a group of miscellaneous cases with edema from hypoproteinemia of relatively short duration. Six patients with burns who had developed edema with low serum proteins from excessive saline administration were treated 11

[11] This patient was treated by Dr. Raymond G. Fillsinger, Buffalo Children's Hospital, Buffalo, N. Y.

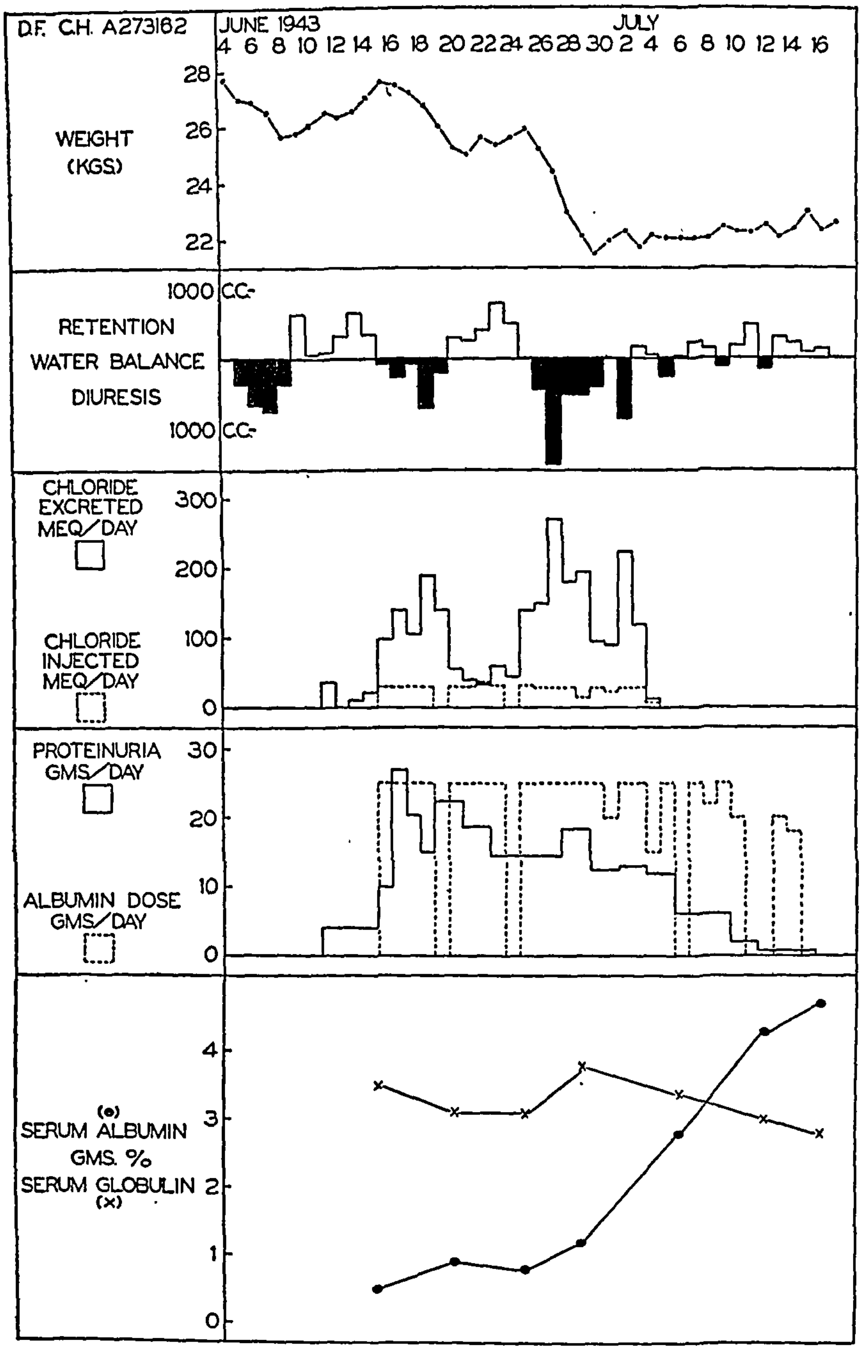

FIG. 10. CLINICAL COURSE OF 8-YEAR-OLD BOY (D. F.) WITH NEPHROSIS
Note failure of serum albumin level to rise appreciably until proteinuria diminished.

to 14 days after injury. The edema was reduced in each, and the serum albumin elevated in all but 1, whose diminished hematocrit reading showed that the plasma volume had probably increased. One patient with marked edema, as a result of the hypoproteinemia which followed a longer period of protein depletion after a very severe burn, received 200 grams of albumin and 1500 cc. of plasma in 6 days. This resulted in a marked diuresis, clinical improvement, and a rise of serum albumin from 1.3 grams per cent with a total protein of 3.1, to 2.3 grams per cent with a total protein of 4.3. Four cases who developed edema during convalescence from intestinal surgery were treated. One of these, who received 195 grams in 7 days with elevation of serum albumin from 2.2 to 3.3 grams per cent, showed a considerable decrease in edema. Another experienced a reduction in edema following 25 grams and complete disap-

pearance after a second injection. One week later the edema had again set in and 25 grams was without effect. A third, receiving 50 grams, lost his edema, the serum protein increasing from 6.2 to 6.6 grams per cent, whereas a fourth case showed no effect on edema, serum protein, or hematocrit following 25 grams. In several patients with hypoproteinemia associated with suppuration, acute hepatitis, and carcinoma, the administration of albumin raised the serum protein level and aided in recovery when the condition was not hopeless. The doses used in these patients were 25 to 50 grams per day, except in a 7-month old baby who received 10 grams per day. In a patient, F. G., with long-standing hypoproteinemia associated with chronic constrictive pericarditis, 490 grams of albumin induced an increase in the retention of nitrogen (Table V; II) but failed to change her clinical condition or to alter the serum albumin level appreciably.

Discussion

Due to the specifications for concentrated normal human serum albumin, which required the clinical trial of each preparation before delivery to the armed forces, we have had a unique opportunity to use large amounts of this material in a few patients with hypoproteinemia. The requirements have, however, limited us to doses of no more than 25 grams per patient per day in most cases and to the treatment of patients in whom rises in temperature could be interpreted. This precluded the treatment of acutely ill patients in whom albumin might have produced more dramatic results and restricted us largely to those chronically ill.

The outstanding finding was that very large amounts of albumin were needed to raise the serum albumin level in patients with chronic hypoproteinemia, whether it was on a basis of malnutrition, altered synthesis as in cirrhosis of the liver, or proteinuria, as in the nephrotic syndrome. Since the amounts necessary to increase the circulating plasma proteins are so large, the use of albumin is chiefly indicated to tide the patient over an emergency. In malnutrition, the diet may be supplemented by protein hydrolysates given by mouth or by vein. However, if the serum proteins fall to edema levels, intensive plasma or concentrated albumin therapy may be of value for a period.

In patients with cirrhosis of the liver with ascites, albumin therapy was found to have little to offer beyond temporary improvement in nutrition. The effect of raising the level of serum albumin on ascites was difficult to assess, but suggested that hypoalbuminemia is not the chief factor in its production.

In patients with severe proteinuria, a condition analogous to plasmaphoresis is set up, whereby the patient loses large amounts of albumin daily, indicating synthesis of plasma albumin and inability to retain it in the circulation. Since a considerable portion of injected albumin is lost in the urine, it is difficult to evaluate the effect of treatment, unless quantitative measurements of protein excretion are made, in order to determine how much albumin is actually retained. This amount seems to vary from one case to another, as Luetscher has shown (26), and at different times in the same patient (Figures 9 and 10). Our best results were obtained in 2 children with nephrosis, in whom the satisfactory clinical response may have been coincidental, or due to either the nature of their disease or to the larger doses given. Further studies, particularly in children, are justified on the basis of our experience, but only when sufficient albumin can be spared from the urgent needs of the armed forces. However, it is evident that enormous doses may be given without clinical improvement, that diuresis does not regularly result from albumin administration, and that when the albumin level in the plasma is temporarily raised by an injection of concentrated albumin, there is a corresponding increase in protein excretion.

Albumin has certain possible advantages over plasma for the treatment of hypoproteinemia. It is usually the deficient plasma protein in these cases. A large dose of protein can be administered in a small volume of fluid, a great asset in children. For the treatment of edematous patients, it could be dissolved in glucose solution instead of in 0.85 or 1.7 per cent sodium chloride, making it possible to administer the needed protein without salt. It should be stressed that concentrated albumin has not been shown to be a diuretic in 25-gram doses in adults. The evi-

dence from a few cases suggests that if 50 to 75 grams are administered in a short time to hypoproteinemic patients, producing a sufficient rise in colloid osmotic pressure with a more prolonged increase in plasma volume, some diuresis and diminution of edema may occur.

In the treatment of hypoalbuminemic patients, several factors must be kept in mind. Large doses are obviously necessary to produce an effect, and it would seem logical to administer a maximum amount of albumin in a minimum period of time. If the albumin is given much faster than it can be removed from the blood stream and metabolized or stored, venous congestion and excessive hemodilution may occur, as indicated by the course of one patient who received 650 grams in 6 days. Our experience suggests that the margin of safety is wide in most patients who do not have cardiac failure, but in attempting to raise the serum protein level rapidly, one should be guided by observations of the degree of venous and pulmonary congestion, and by determinations of hematocrit or hemoglobin level, in order to avoid overloading the circulation.

In addition, there are several considerations which should influence further studies on hypoproteinemic patients. It is quite possible that the various plasma globulin fractions may prove more useful in the treatment of certain forms of protein deficiency than albumin, since the fractions of plasma differ in amino acid composition, as shown by Brand (27), and in ability to promote plasma protein production in animals according to Cannon (28). Furthermore, in chronic protein depletion, the regeneration of tissue proteins is probably more important than the synthesis of the more readily measured plasma proteins.

It seems clear from our experience that concentrated albumin should have a useful place in therapeutics not only for the emergency treatment of shock, but for the treatment of a number of conditions in medicine and surgery, characterized by deficiency of serum albumin. Although we have had little opportunity to treat the acute and readily reversible types of hypoproteinemia, what experience we have had indicates that it is in this group of patients that albumin has most to offer. The rather disappointing results in the patients with chronic hypoproteinemia due to the nephrotic syndrome or to cirrhosis of the liver is scarcely surprising and is in keeping with the observations of others who have had the opportunity to use large amounts of plasma in these conditions. Further careful studies on the possible therapeutic usefulness of albumin should be carried out, when the armed forces have been supplied with sufficient albumin to exceed their needs for the treatment of shock.

Summary of Part III

1. Evidence has been presented that concentrated human serum albumin is utilized like native serum albumin by hypoproteinemic patients.
2. Very large amounts of albumin were needed to raise the serum albumin level in patients with chronic protein depletion, whether on a basis of malnutrition, altered synthesis, as in cirrhosis of the liver, or chronic protein loss, as in nephrosis.
3. Only a small portion of the albumin retained could be accounted for in the circulation, and thus the major portion was presumably stored.
4. Although albumin did not appear in the urine in significant amounts after injection into patients with normal kidneys, a large part of that injected into 2 patients with the nephrotic syndrome was excreted.
5. It was possible to raise the level of serum albumin and decrease the level of serum globulin in patients with cirrhosis of the liver by repeated albumin injections, but this merely served to improve the patient's state of nutrition temporarily.
6. No conclusions are possible concerning the effectiveness of albumin in the treatment of nephrosis, but the results have been sufficiently encouraging in children to justify further careful investigations.
7. Although we have had few opportunities to treat cases of acute hypoproteinemia, the results suggest that albumin may ultimately prove most useful in this group of cases.
8. The main limiting factor in the rapid elevation of the serum protein level by albumin administration is the capacity of the circulation to adjust to increases in blood volume. Observations of the degree of venous and pulmonary

congestion and hemoglobin or hematocrit measurements should be used to guide therapy.

FINAL SUMMARY

Concentrated human serum albumin was developed to meet the needs of mobile military groups for a compact, stable, quickly available blood substitute for emergency use.

Part I deals with its use in shock. The standard Army and Navy package contains 25 grams of albumin in 100 cc. of diluent, and is equivalent to 500 cc. of citrated plasma in osmotic effect. When injected, it draws fluid rapidly into the circulation and has been successfully used in civilian hospitals to restore blood volume in 91 cases of shock due to trauma, hemorrhage, operations, and burns.

In Part II, the safety of albumin is discussed. Properly processed albumin does not produce reactions. Pyrogenic reactions were encountered in trials of certain lots, which were therefore rejected until the albumin was reprocessed to a satisfactory state. No evidence of sensitization to either native or heated albumin (50° C. for varying periods) was found. Neither clinical nor pathologic evidence of damage from repeated albumin injections was observed, except in one case in which pulmonary edema was attributed to overdosage (650 grams of albumin containing 1 : 10,000 merthiolate in 6 days).

In Part III, some data on the effects of repeated albumin injections in a small group of hypoproteinemic patients are reported. Albumin did not appear in the urine after injection unless there was proteinuria, nor was its injection followed by an increase in urinary non-protein nitrogen in hypoproteinemic patients, indicating assimilation, since only a small portion of the injected albumin could be accounted for in the circulation. Very large amounts given in doses of 25 grams daily were needed to produce an appreciable rise of serum albumin concentration in patients with chronic hypoproteinemia. In cirrhosis of the liver, albumin raised the serum albumin level but did not produce lasting benefit. In nephrotic patients, the injected albumin was largely excreted in the urine as protein; diuresis did not regularly result from albumin treatment but occurred coincidentally in 2 cases. There was little opportunity to treat patients with acute hypoproteinemia, but the results in a few cases suggest that albumin ultimately will be most useful in this group.

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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

VIII. CLINICAL USE OF CONCENTRATED HUMAN SERUM ALBUMIN IN SHOCK, AND COMPARISON WITH WHOLE BLOOD AND WITH RAPID SALINE INFUSION [1,2]

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The present report is concerned primarily with the use of concentrated human albumin solutions, produced from pooled normal human plasma by the method of Cohn and co-workers, (1), in the treatment of shock in man.[3] This work represents a part of a more general study (3) of the circulation in human cases of shock, which has been in progress at Bellevue Hospital, New York City, during the past two years.

In addition, the therapeutic effects of concentrated human albumin will be compared with the effects of whole blood, and of rapid intravenous saline infusion.

Before presenting the results of this investigation, it may be well to define briefly two separate aspects of shock therapy, since the differentiation of these provides the basis of our comparison of the three types of treatment used.

For successful treatment of clinical shock, two things are essential; first, to restore the failing circulation; second, to restore the failing (anoxic) tissues. The circulation can be restored, in most instances, by replacing the deficit in blood volume. The tissues, however, can be restored only by abundant oxygen supply and carbon dioxide removal. For this purpose, the blood must contain adequate amounts of hemoglobin.

To put the matter in its simplest terms, the heart could pump plasma, or even salt solution around the circulation, but neither saline nor plasma will keep tissues alive.

If the blood is anemic, oxygen will be adequately supplied to the tissues only if the cardiac output or total blood flow is proportionately increased above normal. For quantitative discussion of this, we have added the concept of arterial oxygen transport, derived from a scheme presented some years ago by Murray and Morgan (4). This will be considered further below.

MATERIAL

Concentrated human albumin therapy has been given in 12 cases of severe bodily injury, which may be classified as follows:

3 cases of multiple compound fractures.
1 case of fracture of the pelvis and rupture of the spleen.
3 cases of profuse external hemorrhage.
2 cases of intra-abdominal injury: one with strangulated hernia, and one with perforation of the duodenum.
3 cases of severe burn.

The product used was a solution of human albumin, 25 grams in 100 cc. In 9 instances, the preparation was albumin in saline (both 0.15 and 0.3 molar NaCl); in 3, albumin in 0.3 molar solution of sodium chloride and sodium acetate; in 1 case, both types of preparation were administered.

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Columbia University, with the collaboration of New York University. Additional support for the research was provided by the Commonwealth Fund and the Josiah Macy, Jr. Foundation.

[2] The products of plasma fractionation employed in this work were developed from blood, collected by the American Red Cross, by the Department of Physical Chemistry, Harvard Medical School, Boston, Massachusetts, under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[3] This study was carried out in order to provide more detailed information on the mechanism of the action of albumin in shock than was possible in the original clinical appraisal (2).

METHODS

The methods of measurement of the circulation, as described in a previous report (3), were used. The following measurements were taken before and at various intervals after injection of the concentrated albumin: (*1*) plasma volume, hematocrit, and serum protein concentration; (*2*) tracings of the pressure contours in the femoral artery; (*3*) right auricular blood pressure, through a long catheter introduced via brachial vein into the right heart; (*4*) simultaneous carbon dioxide content, oxygen content and capacity and pH_s in the arterial blood, and in the mixed venous blood (sampled from the right auricle); (*5*) oxygen consumption and pulmonary ventilation; (*6*) blood lactate. From these measurements, the following data were obtained by calculation: (*1*) total blood volume; (*2*) mean arterial blood pressure; (*3*) oxygen arterio-venous difference; (*4*) cardiac output and stroke volume; (*5*) peripheral vascular resistance; (*6*) pCO_2 and pH_s arterio-venous differences; (*7*) alkali reserve (T40); (*8*) effective arterial oxygen transport to the tissues. In 2 cases, renal circulation was studied by clearance methods.

Because of their particular importance in this study, methods used in determining plasma volume changes and total blood volume changes, and in calculating the amount of albumin retained in the circulation will be briefly outlined.

Plasma volume was measured before and at suitable intervals after injection of the albumin, by the dye dilution method with T-1824 (3), the initial concentration of the dye being obtained by extrapolation of a time-log concentration curve. The corresponding total blood volumes were calculated directly from the arterial hematocrit

TABLE I

Effects of concentrated human albumin injection upon blood volumes in 12 cases of shock

Patient	Type of injury	Hemorrhage between determinations	Albumin				Total crystalloid solutions	Serum protein concentration		Hematocrit		Plasma volume		
			Variety	Injected	Retained	Time after injection		Before	After	Before	After	Initial total	Observed increase†	Increase per gram of albumin retained
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
				grams	*grams*	*hours*	*cc.*	*grams per cent*	*grams per cent*	*per cent cells*	*per cent cells*	*cc.*	*cc.*	*cc.*
S. H.*	Bilat. frac. tibiae	+	S	34	19 23	2:45 5:15	795 300	6.3	6.1 6.1	37	28 28	2210	460 535	24.2 23.2
W. C.	Fr. humerus, ribs, pelvis, tibia, pnx.	+	S	63	35	1:45 5:15	1150 345	4.5	5.2 5.4	34	27 28	2070	400 370	11.4
H. S.	Fr. pelvis, ankles, bilateral fr. os calci	+ +	A A	50 46	34 27	1:30 2:15	350 365	5.0 5.3	5.3 5.6	32 20	20 15	1390 1940	640 410	18.8 15.2
T. L.	Rupt. spleen, fr. pelvis	++	S	66	8	3:15	1285	6.0	5.8	41	27	1700	400	50.0
B. S.*	Lacer. of neck, wrist	0	 S	47	41 25	3:15 8:30 36:00	1355 750 0	6.8	6.1 6.1 5.7	38	29 29 30	2160	1075 1185 835	26.2 33.4
S. C.*	Rupt. varicose veins	0	S	70	62 68	3:15 25:15	1150 0	5.1	5.4 5.2	36	26 23	1960	1140 1380	18.4 20.3
W. K.	Lacer. of radial artery	0	A	46	36	1:00	310	5.3	5.7	36	31	3010	480	13.3
M. C.	Perforated peptic ulcer	0	S+A	72	38	2:30	440	6.0	5.8	64	48	1330	760	20.0
M. G.	Strangulated hernia	0	S	20	9	1:00	655	9.0	8.5	48	43	2160	300	33.4
J. O.*	2nd degree burns, 20 per cent B.S.	0	S	95	73 24	2:15 12:15	2235 300	6.5	6.3 5.7	60	45 52	1510	1290 750	17.7 31.2
L. V.	2nd, 3rd degree burns, 20 per cent B.S.	0	S	44	19	3:30	835	4.9	4.7	45	34	1560	635	33.4
J. C.*	2nd degree burn, 20 per cent B.S.	0	S	46	31	6:00	185	5.4	5.7	47	39	1610	480	15.5

* Determination by Howe-Kjeldahl method.

† After correction for blood sampling between determinations.

readings. It should be noted that the total blood volume so calculated assumes that the hematocrit in large and small blood vessels is the same. In calculating plasma volume and total blood volume changes, figures were corrected for blood samples taken in the interval. Serum protein concentrations were calculated from the serum specific gravity, obtained by the falling drop method. The total circulating protein was calculated as the product of plasma volume times the serum protein concentration. Protein loss due to sampling and in some instances to bleeding, was calculated according to a method described elsewhere (3). The net protein increase between the initial and any subsequent measurements was then assumed to measure the amount of albumin retained. To test the validity of this assumption, in 5 of the 12 cases studied, the total protein nitrogen and albumin/globulin ratio were determined by the combined Kjeldahl and Howe technics before and after injection of a known quantity of concentrated albumin. It was found in each instance that the increase in total circulating protein was entirely accounted for by the increase in the albumin fraction; the total circulating globulin remained constant, although its concentration decreased. It has been assumed that each 100 cc. of concentrated albumin solution contained 25 grams of albumin.

RESULTS

I. Influence of concentrated human albumin therapy upon plasma volume, hematocrit, and serum protein

Table I summarizes the results of the 12 cases reported. In the first 2 columns, they are classified according to type of injury. Under the third column, note is made whether or not further blood loss occurred, during albumin therapy. This was in all instances confirmed by operation, autopsy findings, or development of a visible hematoma. In the next columns (4, 5, 6, 7, 8) are tabulated the variety of preparation used, amount of albumin injected, amount of albumin retained, amount of crystalloid solution introduced intravenously between blood volume determinations, and interval of time between studies. A double entry is made of Patient H. S., who received albumin in 2 separate doses and was thus studied twice.

In columns (9, 10) under serum protein concentration, the figures entered were obtained at the time of dye injection. In the column (14) "observed increase" in plasma volume, the figures are net changes, from direct reading, after allowance was made for intervening blood samplings.

The data reported in Table I may be summarized as follows:

In the cases of skeletal trauma and hemorrhage with evidence of further bleeding after albumin therapy had been started, there was a sizeable loss of albumin. In cases of burns and peritonitis, with exudation of protein-rich fluid, there was also considerable loss of albumin.

On the other hand, in the 3 cases (B. S., S. C., W. K.,) where there had been no further blood loss and no protein-rich exudation, the several determinations in the table suggest that the albumin was almost wholly retained, and later disappeared slowly from the circulation. On the basis of available data, there appeared to be no difference between the "saline-treated" (S) and "acetate-treated" (A) albumin from the point of view of the retention in the blood stream.

In general, the serum protein concentrations tended to remain about the same before and after albumin administration (Figure 1). Since the average serum protein concentration before treatment was less than normal, this suggests that the blood was diluted, by fluid retention or absorption from tissues, to this previously existing level. In the individual case, however, there was no constant correlation between the initial level of serum protein and the degree of dilution per unit dose of albumin. The amounts of intravenous saline may have contributed to this dilution. But here again there was no correlation between degree of blood hydration following treatment, and the amount of saline that had been given. The smallest volumes of saline given were 185 cc. in a case of burn, and 310 cc. in a case of hemorrhage. There was no seriously dehydrated patient, so that our series of cases probably did not test the limiting capacity of the concentrated albumin to draw fluid from tissues into the blood stream.

The average amount of blood volume increase per gram of albumin *retained* was 23 cc. This compares with 17.4 cc. per gram of albumin *given*, for the series of normal subjects reported by Heyl, Gibson, and Janeway (5) and with 18 cc. per gram obtained by Scatchard, Batchelder and Brown (6) by *in vitro* measurement of osmotic pressure.

In the cases showing hemodilution, the total

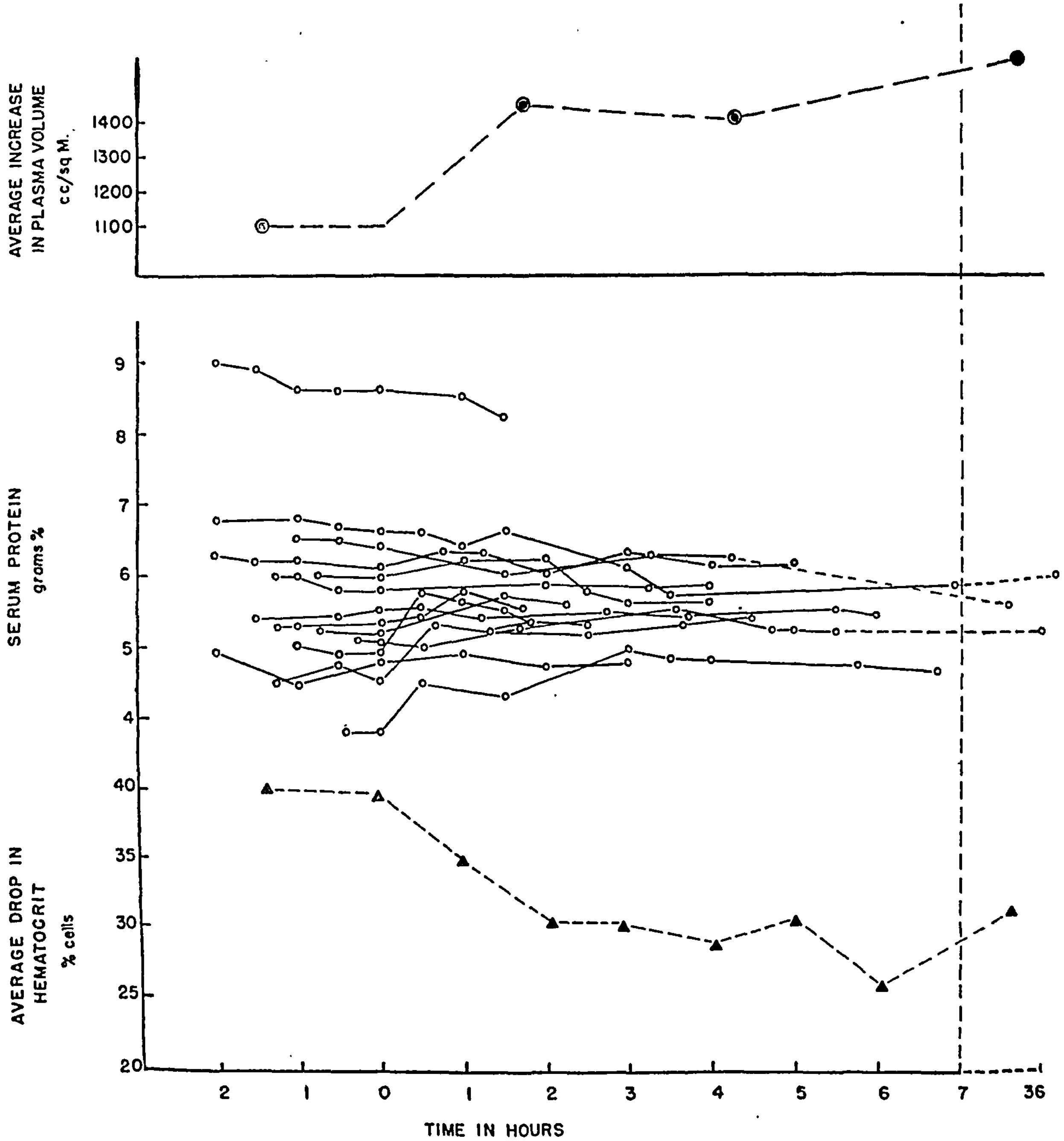

FIG. 1. PLASMA VOLUME, SERUM PROTEIN CONCENTRATION AND HEMATOCRIT VALUES IN 12 CASES OF INJURY WITH SHOCK, BEFORE AND AFTER TREATMENT WITH CONCENTRATED HUMAN ALBUMIN

The albumin was given at zero time on the horizontal scale.

blood volume calculations before and after treatment accounted very satisfactorily for the total red cell volumes. Calculation in the 3 cases of external hemorrhage, for example, gave nearly the same red cell volumes before and after albumin therapy; in the 3 cases that had hemoconcentration, however, there were considerable discrepancies, apparent losses of 160 cc., 180 cc., and 280 cc. of red cells per square meter of body surface being calculated after treatment, when there had been no evidence of further hemorrhage. This discrepancy has been encountered before in the presence of hemoconcentration and is being studied further. It should also be noted that 2 cases (W. C. and B. S.) received small amounts of whole blood during the time of initial study, since the condition of the patient was poor and it was felt that it was necessary to give some immediate therapy to sustain the circulation at this time.

The increase in blood volume brought about by the albumin injected was still maintained at the end of the period of observation, usually 6 hours; the plasma protein concentrations showing little change. This is well illustrated in Figure 1.

II. Effect of concentrated human albumin therapy upon the dynamics of the circulation and arterial oxygen transport

In appraising various treatments of peripheral circulatory failure, recent emphasis has rightly shifted from improvement in arterial blood pressure, to the restoration of blood volume, thereby improving auricular pressure (an approximate index of venous return) and cardiac output.

For relief of tissue anoxia, however, the essential requirement is an increase in the amount of oxygen supplied to the tissues. The "arterial oxygen transport," or cardiac output times oxygen content of arterial blood, expresses this function (4). Murray and Morgan used for this function the term "oxyhemoglobin flow."

In Table II are given individual measurements of the circulation and of arterial oxygen transport in 11 cases, before and after albumin treatment. With 2 exceptions, these cases presented evidence of severe peripheral circulatory failure, before treatment.

In 9 of the 11 cases, significant amounts of albumin were retained in the circulation following injection, with an associated increase in plasma volume. In all these cases, there were: (*1*) a rise in auricular pressure; (*2*) considerable increase in cardiac output; (*3*) increase (though

TABLE II

Measurements of the circulation and of oxygen transport in 11 cases before and after treatment with concentrated human albumin

Patient (body surface)	Diagnosis	Degree of shock	Fluid replacement: Albumin retained	Fluid replacement: Crystalloid solution	Blood volume: Plasma*	Blood volume: Total	Blood volume: Hemat.	Auricular pressure	Cardiac output	Arterial mean press.	Peripheral resistance	Arterial oxygen transport	O_2 consumption
Normal values			0	0	600	2800	43	35	3.2	95	1300	550	150
			grams	*cc.*	*cc. per sq. M.*	*cc. per sq. M.*	*per cent*	*mm. H_2O*	*L. per minute per sq. M.*	*mm. Hg*	*dynes cm.$^{-5}$ second*	*cc. per minute per sq. M.*	*cc. per minute per sq. M.*
S. H. (1.70)	Bilat. fract. tibiae	+	19	795	ā 1300 p̄ 1510	2060 2100	37 28	+10 +27	3.13 4.21	80 77	1160 810	429 493	146 177
W. C. (1.75)	Fract. humerus, ribs, pelvis, tibia, pnx.	+++	35	1150	ā 1180 p̄ 1440	1800 1990	34 28	+2 +6	2.17 4.29	59 66	1240 700	245 408	187 274
H. S. (1.63)	Fract. pelvis, ankles, bilateral fr. os calci	+++	61	350	ā 850 p̄ 1400	1260 1670	32 16	−3 +17	1.24 4.24	38 88	1500 1020	146 271	114 131
T. L. (1.80)	Rupt. spleen, fract. pelvis	+++	8	1285	ā 950 p̄ 1090	1600 1490	41 27	−4 +1	1.97 2.36	52 64	1170 1205	294 252	177 168
B. S. (1.60)	Lacer. of neck, wrist	+	43	1355	ā 1350 p̄ 1970	2170 2740	38 29	−26 −8	2.69 4.33	100 100	1840 1160	342 459	137 160
S. C. (2.00)	Rupt. varicose veins	++	62	1150	ā 980 p̄ 1500	1530 2030	36 26	+4 +59	2.00 4.50	74 91	1420 810	270 491	164 212
W. K. (1.84)	Lacer. of radial artery	++	36	310	ā 1690 p̄ 1870	2560 2710	37 31	+20 +46	2.40 3.31	39 45	710 590	353 427	175 179
M. C. (1.69)	Perforated peptic ulcer	+++	38	440	ā 790 p̄ 1210	2180 2320	64 48	−18 +65	1.33 2.73	90 106	3200 1830	298 470	152 139
M. G. (1.56)	Strangulated hernia	+++	9	655	ā 1440 p̄ 1600	2830 2810	49 43	+24 +22	1.65 1.87	79 91	2450 2490	312 326	165 170
J. O. (1.81)	2nd, 3rd degree burns, 20 per cent B.S.	++	73	2235	ā 840 p̄ 1500	1990 2740	58 44	+5 +105	2.09 6.85	90 104	1910 620	417 1110	119 295
L. V. (1.67)	2nd, 3rd degree burns, 20 per cent B.S.	++	18	835	ā 930 p̄ 1180	1700 1790	45 34	−5 +14	1.83 4.24	79 65	2070 734	322 560	152 165

* As calculated directly from optical density readings.

less consistent) in arterial blood pressure; and (4) decrease in peripheral vascular resistance. These changes were associated with marked clinical improvement. No unfavorable side-effects were noted.

In 2 cases, T. L., who continued to bleed profusely, and M. G., who lost plasma protein into peritoneal exudate, there was no appreciable retention of albumin in the circulation and no increase in blood volume, in auricular pressure, or in cardiac output. Clinically, these patients were not improved.

One statistical value in this series is of interest. The coefficient of correlation between the amount of albumin retained in the circulation, and the increase in cardiac output, had the extraordinarily high value of $r = +0.928$ ($P < 0.0004$).

So far as oxygen supply to the tissues is concerned, it will be seen from the last 2 columns in Table II, that in all cases which improved, both an increase in oxygen consumption and an effective increase in arterial oxygen transport were found. However, it will also be noted that the cardiac output, in 7 of the 9 cases, was actually increased above normal values.

III. Description of individual cases

This first patient, with extremely severe skeletal trauma, in profound shock, received very large amounts of concentrated human albumin.

Case 1. H. S., a 48-year-old white female, jumped from a third story window, and sustained bilateral fractures of os calci, fracture of the ankles, of the pelvis through the sacrum and of the transverse processes of several lumbar vertebrae. She was first admitted to the psychopathic ward and one hour later was transferred to the emergency ward.

The first series of measurements was completed 3½ hours after injury. The patient was mentally depressed, answering questions clearly, however, and complaining of thirst. There was marked pallor of the skin, and coolness of the extremities. Sweating of the forehead and cyanosis of the lips and ears were noted. Peripheral veins were collapsed, and upon exposure the median basilic vein was found in a state of active constriction. As seen in Figure 2, the arterial blood pressure, which was low on admission, remained unchanged. There was marked reduction in the blood volume to about 50 per cent of normal, with a hematocrit of 32 per cent, and plasma protein level of 5 grams per cent. Other hemodynamic findings were: low auricular pressure, and a very low cardiac index and stroke volume, respectively reduced to 1.24 liters per minute per square meter of body surface, and 18 cc. per beat. The oxygen arterio-venous difference of 96 cc. per liter of blood circulating was high and the oxygen consumption, low. As observed in other cases studied relatively soon after injury, the arterial blood pH_s was only moderately decreased, to 7.32, but the pH_s arterio-venous difference of 0.06 was abnormally large. Ventilation was slightly elevated, the pCO_2 arterio-venous difference was 9 mm. Hg. Blood lactate was 23 mgm. per cent. The arterial blood oxygen saturation was normal. There was complete anuria, suggesting greatly reduced renal circulation (3).

Two 100 cc. ampules (50 grams) of concentrated human albumin in saline-sodium-acetate solution and an additional 150 cc. of an isotonic saline solution were injected in the next half hour. Although pallor and coolness of extremities still persisted, the general appearance was then somewhat improved. A second series of measurements, completed about 2 hours after the first, showed: (*a*) a rise in arterial blood pressure and (*b*) an increase of plasma volume of 340 cc. per square meter of body surface, with a significant reduction in total red cell volume; as previously discussed, there was indication that active bleeding was still taking place. The total blood volume had increased by about 20 per cent over the first measurement. The hematocrit was down to 20 per cent; the plasma protein had increased slightly (see Table I). There was significant increase in auricular pressure and an increase in cardiac index to 2.20 liters per minute per square meter of body surface. The oxygen consumption was still low, but the oxygen arterio-venous difference had diminished to 51 cc. per liter of circulating blood. With the improvement of circulation, arterial pH_s returned to the normal figure of 7.39 and the blood lactate decreased to 13.5 mgm. per cent. Urine flow began but measured only 0.06 cc. per minute, indicating some increase in renal blood flow and filtration, although if one may judge from a previous study on correlation between urine flow and clearance (3), the renal circulation must still have been small.

During the next hour, 2 more ampules (50 grams) of concentrated human albumin and 180 cc. of isotonic crystalloid solution were injected. The general appearance improved considerably, although pallor of the skin was still present. A hemic murmur became audible, loudest at the pulmonic area. After an interval of 1 hour, a third series of measurements was taken. The mean arterial blood pressure had increased to 88. The plasma volume was almost back to normal. No further bleeding had apparently taken place (see Table I), but because of the previous loss of red blood cells the total blood volume was still low. The hematocrit had reached the very low level of 16 per cent; the plasma protein had increased very slightly (see Table I). Obviously, a large amount of fluid had been shifted from the extravascular compartment into the circulating blood. Auricular pressure was maintained at an almost normal level and the cardiac index had increased to 4.24 liters per minute per square meter of body surface, a value appreciably above normal. The oxygen arterio-venous difference was low at 31 cc. per liter of blood and the oxygen consumption had increased by about 15 per cent. The alkali reserve (T40), slightly diminished at the time of the first studies, was now somewhat above the upper

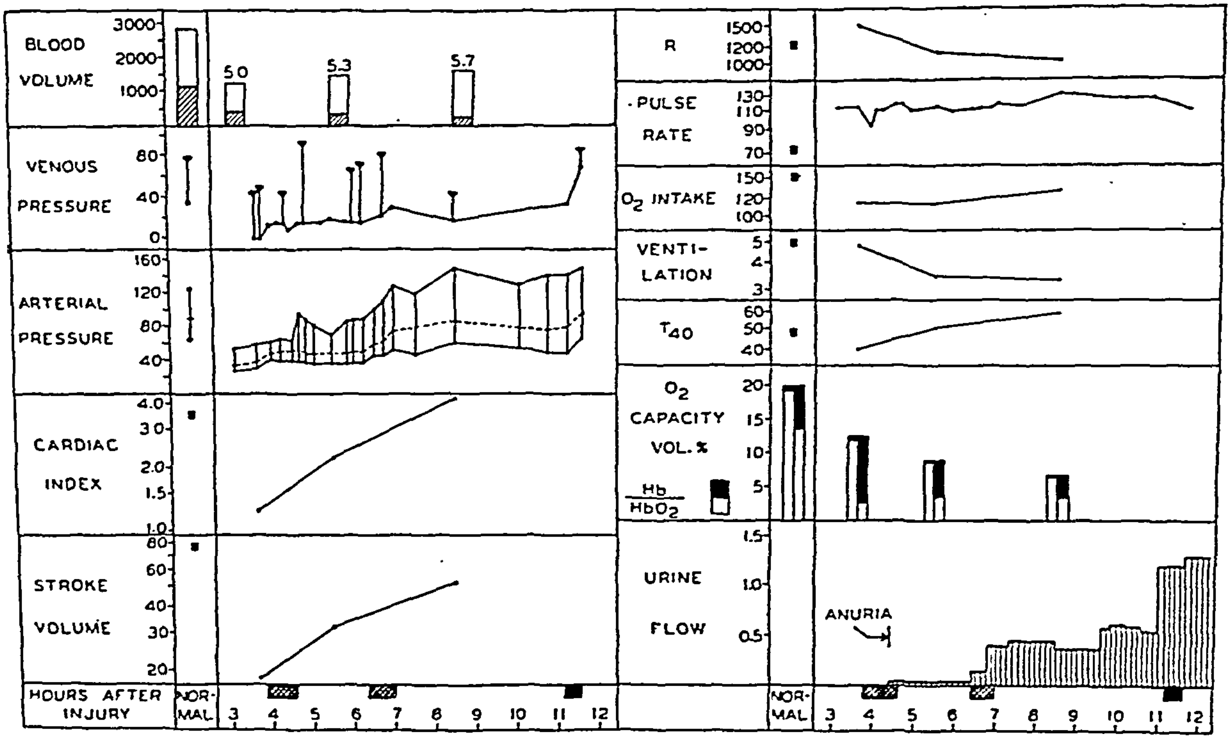

FIG. 2. MEASUREMENTS OF THE CIRCULATION IN THE COURSE OF TREATMENT OF H. S., A CASE OF SEVERE SHOCK DUE TO MULTIPLE FRACTURES

In blood volume section, unshaded blocks = plasma volume cc. per sq. M. of body surface; shaded blocks = red cell volume; figures at top of columns = serum protein per cent. In venous pressure section, triangles = arm venous pressure, mm. of H_2O; solid dots = right auricular pressures. Arterial pressures are recorded as systolic, diastolic, and mean. Cardiac index = cardiac output as liters per minute per sq. M. of body surface. R = peripheral resistance. O_2 intake = oxygen consumption in cc. per minute per sq. M. of body surface. Ventilation = pulmonary ventilation in liters per minute per sq. M. of body surface. T40 = whole blood CO_2 content at 40 mm. CO_2 tension. The double columns in the O_2 capacity section represent: on the left, arterial blood, with solid block indicating reduced hemoglobin, unshaded block oxygenated hemoglobin as cc. oxygen per 100 cc. blood; the right half-column indicates similar values for mixed venous blood.

The symbols nearest the scales are normal values, for comparison. At the bottom is a time scale indicating hours after injury. The cross hatched blocks at the bottom are the times when albumin was administered; the solid block, whole blood transfusion.

level of normal range. Following the second dose of albumin, the urine flow increased to 0.4 cc. per minute, parallel to the improvement in general circulation during this time. About 1½ hours after the third series of general measurements, inulin and p-aminohippurate clearances were measured and found to average about 75 cc. and 500 cc. per minute, respectively, at a time when the mean arterial pressure was 78 mm. Hg. These are approximately 65 per cent and 80 per cent, respectively, of normal.

Because of the very low hematocrit, 500 cc. of blood were transfused before transferring the patient to the operating room. From then on, she did quite well, and eventually recovered. Renal plasma clearances of inulin and hippurate were measured again 28 hours after injury and were found to be well within the normal range.

Besides exhibiting the usual findings of profound shock, due to loss of blood following multiple fractures, this case presented the following interesting features: (*1*) the large increase of plasma volume following injection of concentrated human albumin (plus a small amount of crystalloid solution); (*2*) the extreme degree of anemia, induced by hemorrhage and precipitated by large shift of water to, or retention in the circulation as a result of albumin therapy; (*3*) a cardiac output increase above normal and out of proportion to total blood volume and oxygen consumption increase; (*4*) the high alkali reserve observed after completion of albumin therapy which is probably accounted for simply on the basis of the large ratio of plasma to red cells, due to the acute anemia, and not related to the alkali (sodium acetate) present in the albumin solution; and (*5*) prompt reversal of the shock-induced renal ischemia.

In brief, this patient, in advanced shock from injury and loss of blood, was brought out of shock by the use of

large amounts of albumin; and passed then into a state of severe but compensated acute anemia, with increased cardiac output, making up in part for loss of hemoglobin.

The second case was one of shock developing in a patient following perforation of a peptic ulcer, with marked hemoconcentration.

Case. 2. M. C., a 43-year-old white female, was first diagnosed as an acute pancreatitis on the basis of: acute onset of severe pain localized to the epigastrium, extreme tenderness around the umbilicus with localized skin cyanosis, rigidity, and rebound tenderness, no x-ray evidence of air under the diaphragm or of intestinal distension, and no history of peptic ulcer. She was first studied 29 hours after the onset of pain.

The patient appeared mentally alert and restless. The extremities were cold, clammy, and cyanotic, the pupils markedly constricted; she had been vomiting. The peripheral venous pressure and the mean arterial pressure were normal, but the pulse pressure was decreased. This contrasted, as shown in Figure 3, with marked changes in other measurements of the circulation, namely, reduction in plasma volume to about 50 per cent of normal; marked hemoconcentration, and slightly low plasma protein concentration; decrease in the total blood volume to about 75 per cent of normal; considerable decrease in auricular pressure; very low cardiac output and stroke volume, respectively, 1.33 liters per minute per sq. M., and 14 cc. per beat. The oxygen arterio-venous difference of 114 cc. per liter of circulating blood was very large and the oxygen consumption, in spite of elevation of the central body temperature, was only 152 cc. per sq. M. The other measurements indicated a large increase in ventilation, with a pCO_2 arterio-venous difference of 9 mm. Hg. The alkali reserve was low, the pH was normal, the blood lactate was elevated to 30 mgm. per cent, the arterial blood oxygen saturation was normal.

Within the next 1½ hours, 3 ampules of concentrated human albumin (75 grams) were injected with an additional 150 cc. of isotonic saline solution. A second series of studies was begun one half hour after completion of the infusion. Cyanosis had then disappeared, the extremities were pink and warm, sweating and restlessness were no longer present. The arterial blood pressure was 130/87. The blood volume studies showed a large increase in plasma volume, a decrease of the hematocrit from 64 to 48, and a small increase in total blood volume. Blood volume determinations recorded an apparent loss from the blood stream of 280 cc. of red blood cells, although there was no evidence of hemorrhage. This discrepancy, already referred to above, accounts for the lack of any larger increase in total blood volume. In spite of this small change in total blood volume, as recorded, the cardiac output had doubled. The peripheral resistance, although still elevated, was much lower than previously. The hyperventilation had partly subsided. The alkali reserve was slightly low, the blood lactate was still approximately 30 mgm. per 100 cc., the pH_s was normal.

Improvement persisted for 4 hours, and as the diagnosis

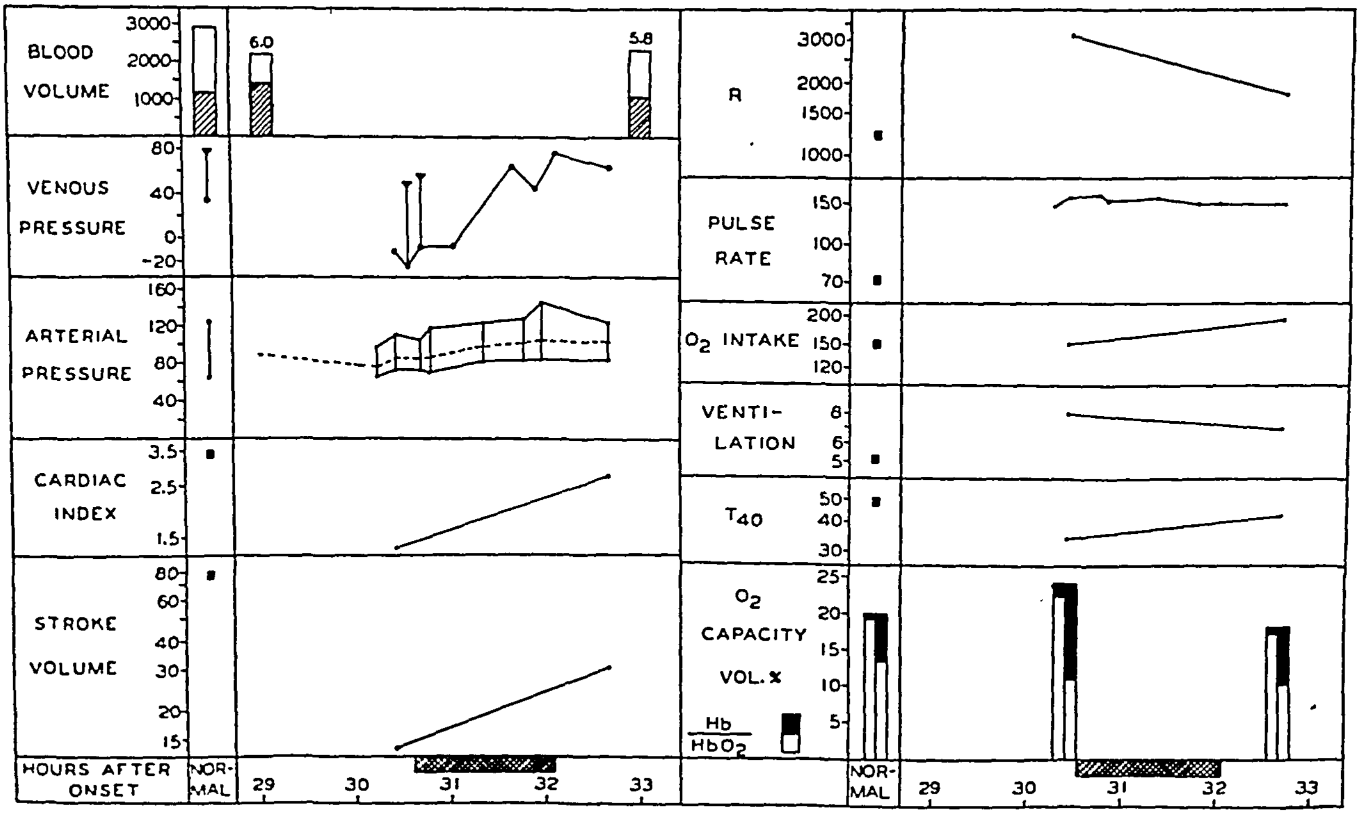

FIG. 3. CASE OF M. C., SEVERE SHOCK FOLLOWING PERFORATED PEPTIC ULCER
For explanation of symbols, see legend under Figure 2.

TABLE III

Measurements of the circulation and of oxygen transport in 10 cases before and after whole blood transfusion

Patient (body surface)	Diagnosis	Degree of shock	Fluid replacement: Blood: Transfused	Fluid replacement: Blood: Retained*	Fluid replacement: Crystalloid sol.	Blood volume: Plasma	Blood volume: Total	Blood volume: Hemat.	Auricular pressure	Cardiac output	Arterial mean press.	Peripheral resistance	Arterial oxygen transport	O2 consumption
			cc.	*per cent*	*cc.*	*cc. per sq. M.*	*cc. per sq. M.*	*per cent*	*mm. H_2O*	*L. per minute per sq. M.*	*mm. Hg*	*dynes cm.$^{-5}$ second*	*cc. per minute per sq. M.*	*cc. per minute per sq. M.*
C. C.	Fract. pelvis	+++				1300	1950	30	0	1.82	22	567	186	164
(1.70)			850	82	200	1950	2790	30	+20	2.66	60	1060	282	190
			500 (pl)											
W. Bl.	Fract. pelvis, torn	+++				1130	1670	32	−5	1.26	36	1250	126	93
(1.82)	urethra		1230	100	1400	1520	2300	34	+18	2.76	72	1145	315	110
M. M.	Fract. pelvis, ribs	+++				1050	1550	32	+16	1.75	46	1430	220	149
(1.47)			1270	83	1010	1460	2240	35	+71	3.95	90	1240	561	216
V. B.	Fract. extrem. skull,	+++				1570	2240	30	+30	1.84	50	1440	201	152
	ribs, lac. scalp,		270	100	650	1770	2530	30	+60	2.91	53	940	294	198
	brain, lung													
J. V.	Fract. limbs, pelvis,	+++				1020	1620	37	−10	1.30	43	1468	190	131
(1.80)	ribs, hematoma		970	68	1330	1330	2080	36	0	3.16	87	1215	427	178
J. E.	G-I hem.	++				1240	2000	36	−31	2.99	60	1145	389	223
(1.41)			380	80		1360	2210	38	−31	2.96	85	1640	438	206
C. F.	Severe lacerations	++				850	1410	40	+24	1.38	60	2200	202	119
(1.58)			690	65	910	1180	1860	37	+64	2.26	100	2240	341	129
H. M.	G-I hem.	++				1380	1960	30	+20	1.96	32	828	229	130
(1.58)			840	100	1380	1680	2540	34	+22	2.24	38	861	289	132
A. H.	G-I hem.	+++				1150	1440	20	+10	1.69	50	1060	134	95
(1.70)			815	100		1350	1850	27	+40	2.57	28	915	275	129
A. S.	G-I hem.	+++				1180	1500	21	+7	1.58	28	2000	134	109
(1.51)			775	100	1145	1620	2210	27	+72	3.35	85	1560	342	126

* Approximate estimate on the basis of calculated increase in circulating red blood cells.

of acute pancreatitis still appeared most probably correct, operation was deferred. Tympanism in the lateral decubitus, suggesting air under the diaphragm, appeared later, but by that time, the patient had taken a turn for the worse and death with hyperthermia occurred 48 hours after the onset of pain.

The autopsy revealed a perforation of the duodenum with acute peritonitis and extension of the inflammation to the right diaphragm and to the pleura.

The special features of this form of shock, developing after rupture of a viscus, with peritonitis, were as follows: (*1*) persistence of a normal mean arterial blood pressure with a small pulse pressure in spite of a considerable reduction in cardiac output, indicating a marked increase in peripheral vascular resistance; (*2*) marked hemoconcentration with only slightly lowered protein content of the serum; (*3*) persistence of a normal pH, in spite of an elevated blood lactate, probably associated with a loss of chloride through vomiting.

From the point of view of concentrated human albumin therapy, the same outstanding features noted before are again present: increase in cardiac output, out of proportion to total blood volume and oxygen consumption increase, and marked decrease in peripheral resistance.

The immediate response to concentrated albumin administration was excellent, with increase in plasma volume, auricular pressure and cardiac output, and decrease in hematocrit and peripheral resistance.

IV. Comparison of concentrated albumin therapy with treatment by (1) rapid saline infusion and (2) whole blood transfusion

(*1*) For purposes of comparison with concentrated albumin therapy, 6 clinical cases have been selected from our data showing the effects on the circulation of rapid intravenous saline

infusion. The group is not very homogeneous, either as regards severity of shock or uniformity of treatment. Three cases received saline only, the amounts varying from 1515 cc. to 1930 cc. One of these was in severe shock, the other 2 were injuries with little or no shock. The other 3 cases received also some whole blood by transfusion, but blood volume studies indicated that little or none of this whole blood was retained in the circulation, as the patients were bleeding. Despite these variations in the conditions of study, the effects of treatment on the circulation were the same in each of these small sub-groups, so that the average results, as given in Table IV, can be said to indicate the trend.

Saline infusion, in these 6 cases, produced a relatively small increase in plasma volume, the figures varying from 10 cc. to 350 cc. per sq. M. of body surface; a small decrease in hematocrit; relatively large increase in cardiac output; small increases in auricular pressure, oxygen consumption, and arterial oxygen transport. In two subjects, repeated measurements carried out an hour or two later (not shown in Table IV) indicated that the cardiac output had begun to fall again; and blood transfusion was then given.

Thus, with saline infusion, one finds, as one would anticipate, a much lesser degree of improvement in the circulation, and less sustained, than with concentrated albumin solution, although on an average, the various changes that do occur are of the same pattern with saline as with albumin. It should be noted that saline infusion does temporarily raise cardiac output, more than one would expect from the small change in blood volume.

(2) Measurements of the circulation in 10 cases of injury, treated with whole blood transfusion, are given in Table III.

There were 5 patients with severe skeletal trauma and 5 with hemorrhage, either internal or external. All were suffering from moderate or severe shock. The amount of blood transfused ranged, in 8 cases, from 680 cc. to 1270 cc.; 2 cases received approximately 300 cc. of blood. Variable amounts of crystalloid solution were also administered. Most of the red blood cells given during transfusion were retained, according to

TABLE IV

Comparison of average hemodynamic response and increase in oxygen transport following treatment with (a) albumin, (b) blood, (c) isotonic saline solution

Type of therapy		No. of cases		Average total blood volume	Average hematocrit	Average auricular pressure	Average cardiac output	Average oxygen consumption	Arterial oxygen transport	Peripheral resistance
				cc. per sq. M.	*per cent*	*mm. H_2O*	*L. per minute per sq. M.*	*cc. per minute per sq. M.*		*dynes cm.$^{-5}$ second*
ALBUMIN*										
Injected alb. Retained alb. Added crystalloid	66 grams 42 grams 852 cc.	6	Initial Final Diff.	1863 2207 +344	36 26	0 +27	2.27 4.15 +1.88	154 189 +35	298 425 +127	1212 845 −367
BLOOD										
Infused blood Retained blood Added crystalloid	809 cc. 88 per cent 921 cc.	10	Initial Final Diff.	1734 2261 +527	31 33	+10 +41 +31	1.76 2.88 +1.12	137 161 +24	201 356 +155	1339 1282 −57
CRYSTALLOID SOLUTION										
Infused	1644 cc.	6	Initial Final Diff.	2070 2234 +164	37 32	+37 +57 +20	2.81 3.94 +1.13	154 170 +16	379 487 +108	1318 1157 −160

* Albumin, 6 cases. Including only those with either skeletal trauma or hemorrhage. See text.

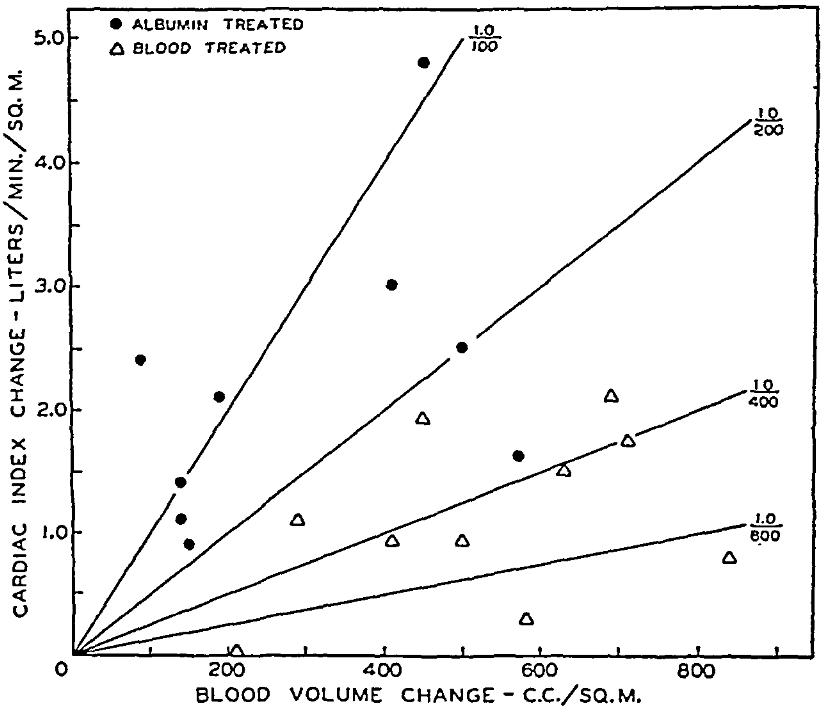

FIG. 4. RELATION OF BLOOD VOLUME CHANGE TO CARDIAC OUTPUT CHANGE, FOLLOWING ALBUMIN (SOLID DOTS), AND WHOLE BLOOD TRANSFUSION (OPEN TRIANGLES)

blood volume determinations (Table III). As shown by blood volume before treatment, as well as by mean arterial and auricular pressures and cardiac output, these cases were on the average in more severe shock than the group in Table II, treated with albumin. Of the blood treated cases, 2 failed to respond. One (J. E.) probably received inadequate treatment. The other (H. M.) had a rise in blood volume but little or none in pressures or cardiac output; this may have been a case of "irreversible shock." The other 8 cases responded satisfactorily to transfusion, with the characteristic changes seen during recovery from shock following restoration of blood volume: rise in auricular and arterial pressures, in cardiac output, and in arterial oxygen transport.

Comparison of the effects of whole blood transfusion with those of concentrated albumin, is given in the average figures of Table IV. In this table are included only the 6 cases in the albumin series which had either skeletal trauma or hemorrhage, since all the whole-blood-treated cases were injuries of these types. This comparison can be only approximate, since the 2 series still differed clinically; but certain trends are apparent. Both series retained roughly comparable volumes in treatment, 712 cc. of blood (88 per cent of 809 cc.), versus 42 grams of retained albumin (equivalent to $42 \times 18 = 756$ cc. of isotonic fluid).

Of the various items in Table IV describing the comparative hemodynamic responses in the 2 groups, the changes in blood volumes, and in auricular pressures, were approximately the same. There was, of course, a decrease in hematocrit, following albumin therapy.

The rise in cardiac output, in the albumin-treated group, was significantly greater than in the whole-blood-treated group. While various factors may have contributed to this difference, an excessive response in cardiac output, in the albumin treated cases, is suggested by the fact that the average figure of 4.15 liters per minute per sq. M. of body surface, after treatment, is nearly one liter per minute greater than the average value for normal subjects by the same technic (3).

This excess cardiac output increase is a favorable compensation, making up for the

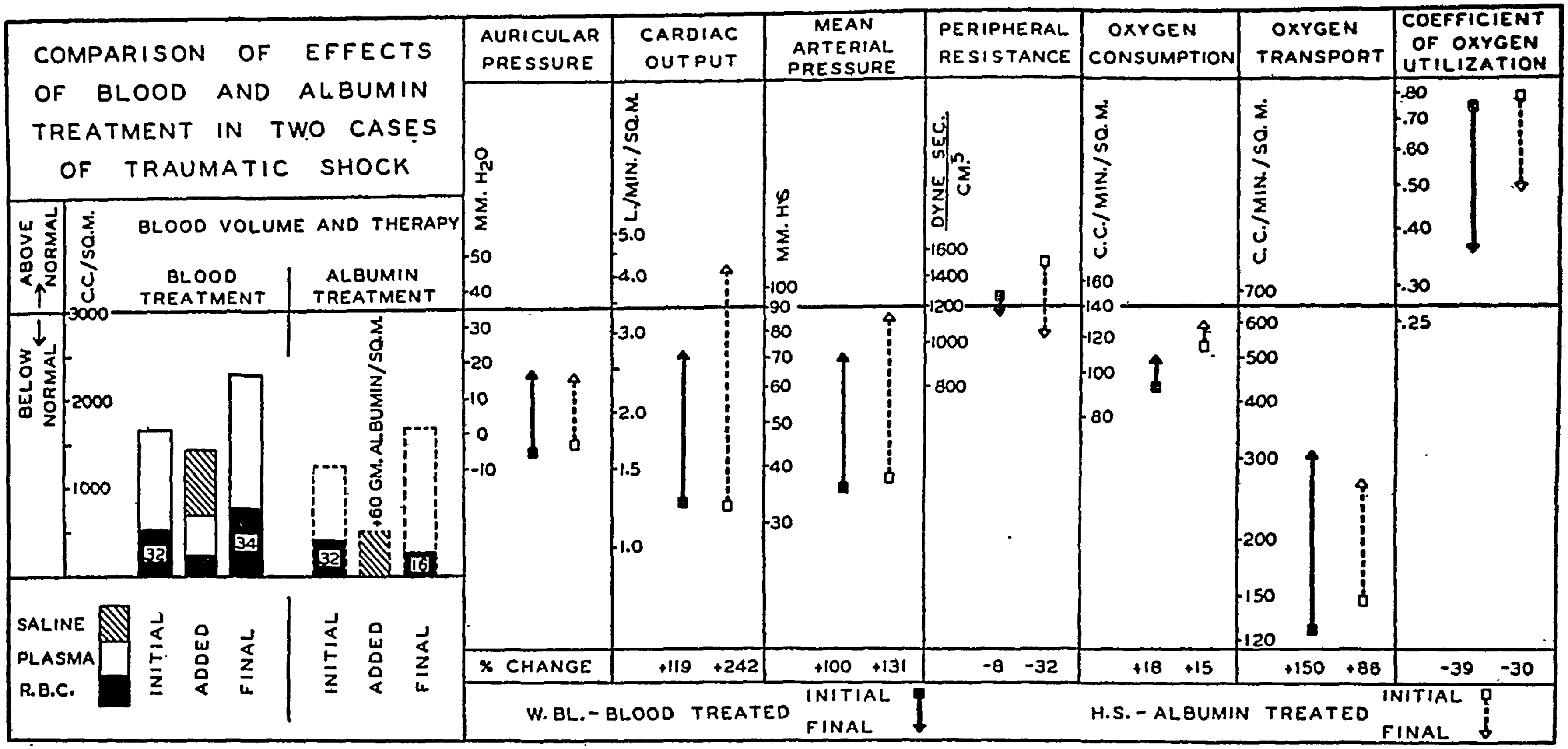

FIG. 5. COMPARISON OF EFFECTS OF TREATMENT IN 2 CASES OF TRAUMATIC SHOCK

With the log scales used as ordinates, the length of the arrows represents the percentage change in the measurement, during recovery from shock.

decreased hematocrit; and resulting in essentially the same increase in average arterial oxygen transport, after treatment, in the albumin as in the whole blood series.

Figure 4 demonstrates the larger cardiac output response in all the albumin-treated cases as compared with the whole-blood-treated cases, in relation to the changes in blood volume.

Increase in total oxygen consumption by the tissues, which is another regular feature of recovery from shock (3), was the same in the 2 groups, and greater than in the saline-treated group, a further indication of restoration of tissue metabolism both by albumin and by whole blood therapy.

A further striking difference between the 2 groups is the decrease in "peripheral resistance" following treatment in the albumin-treated cases, whereas the blood-treated cases showed no change in this function. The function of peripheral resistance, which is essentially the drop in blood pressure per unit of blood flow per second, is measured by the ratio, mean arterial pressure to cardiac output per second. In practical terms, a lowering of peripheral resistance indicates either a decrease in blood viscosity or a widening of blood vascular channels, or both.

To give more emphasis to the different modes of action of concentrated albumin and whole blood, measurements in 2 individual cases, one treated with whole blood transfusion, the other treated with concentrated albumin, are compared in Figure 5. Both cases were almost identical before treatment with regard to the initial degree of shock, amount of plasma and total blood volume loss, auricular pressure, and cardiac output and arterial oxygen transport. The following differences are revealed after treatment: (*1*) With the same increase in oxygen consumption, total blood volume, and auricular pressure, the cardiac output increase in the albumin-treated case was larger, and the increase in arterial oxygen transport was less, than in the whole-blood-treated case. (*2*) There was a considerable decrease in peripheral resistance and in hematocrit in the albumin treated case.

DISCUSSION

The data obtained in the present investigation indicate that concentrated human albumin solution is an effective agent in the treatment of acute traumatic shock in man.

The albumin injected into the circulation is largely retained, providing no further bleeding or exudation of plasma occurs. It is still remaining in the circulation at the end of 6 hours. The albumin retained holds in the blood stream amounts of fluid approximately comparable to its osmotic activity. Thus, in our series, 1 gram of albumin retained resulted in an average increase of plasma volume of 23 cc. This fluid may have been drawn into the blood from the tissues, or may have been simply retained, if additional crystalloid solution was also administered. No unfavorable side effects have been noted from the use of this preparation.

The increase in plasma volume produced by the injection of albumin effects a marked improvement in the circulation. Specifically, all the primary changes associated with recovery from acute shock (3) are observed: increase in right auricular pressure (venous return), in arterial pressure, and in cardiac output. Clinically, the patients were correspondingly improved.

Comparison of the effects of albumin with those of rapid saline infusion have shown that the two are qualitatively similar; but, as would be expected, saline produces a much smaller and more transitory increase in blood volume, and the increase in cardiac output with saline infusion, while considerable, is not sustained.

In comparing albumin therapy with that of whole blood, it is important to recognize that recovery of tissues from the state of shock requires restoration of oxygen transport to the tissues by the circulation; in other words, adequate circulation of. hemoglobin. A normal total blood flow or cardiac output, does not provide normal oxygen transport if the hemoglobin concentration is low. As a measure of oxygen supplied to the tissues, one may use the total arterial oxygen transport, which is actually the cardiac output times the total oxygen content of the arterial blood. This determines the

total amount of oxygen brought to the tissues per unit of time.

In shock due to skeletal trauma or hemorrhage, the reduction in blood volume is regularly associated with *hemodilution* (3). By giving whole blood in such cases, both the blood flow and the amount of hemoglobin which is circulated are increased.

By giving albumin (or plasma), the arterial oxygen transport is restored to normal only if the cardiac output is correspondingly increased above normal. Our figures show that this is actually what occurred in the cases treated in the present series (Table IV). As the available hemoglobin becomes progressively less, for example in continued hemorrhage, there will, of course, be a limit in the capacity of the heart to produce the compensatory increase in blood flow. Case H. S., presented in detail, was probably near this limit.

In brief, after treatment with albumin or plasma, such a patient has recovered from shock, but is still suffering from acute anemia.

The physiological adaptation here is, in fact, that which has long been known to exist in clinical anemia (7, 8). The ultimate effect, also, must be the same, *i.e.*, eventually the heart and circulation can no longer compensate and cardio-circulatory failure will occur (8). In cases recovering from shock, this danger will be greater in the presence of any additional strain, such as operation, or if infection, or other complication, subsequently develops. The use of whole blood or red cells, as soon as available, would thus be logically indicated as additional treatment even when clinically the circulation seems to be restored to normal.

In cases of shock associated with hemoconcentration, as in abdominal injuries and burns, the effects of concentrated albumin administration were favorable, reducing the hematocrit, increasing plasma and blood volume, and restoring or improving the circulation in all but one instance.

In the process of recovery from shock, it is of interest that auricular pressure, arterial pressure, and cardiac output tend to return to normal at a time when the vascular bed is still reduced, as shown by a blood volume not yet restored to normal.

SUMMARY

1. Concentrated human serum albumin solution has been administered in 12 clinical cases of traumatic injury in varying degrees of shock, with measurements of the circulation before and after this treatment.

2. In patients who were not actively bleeding, or losing plasma into burned tissues or peritoneum, the albumin was well retained in the blood. In 9 cases (including 3 burns), an average of 62 grams of albumin was given and an average of 43 grams retained. The albumin tended to remain in the circulating blood for at least 6 hours, when there was no continued blood or plasma loss at the site of injury.

3. Albumin therapy was effective in producing recovery from shock. It increased right auricular pressure, arterial pressure, and cardiac output.

4. Compared with treatment by whole blood transfusion, albumin therapy brought about a relatively larger cardiac output during recovery from shock.

5. In cases of shock due to skeletal trauma or to hemorrhage, where *hemodilution* is regularly found, this increased cardiac output is a compensatory effect, since the tissues can receive adequate oxygen only by more rapid circulation of the diminished amount of hemoglobin in the blood.

6. The persistence of acute anemia in many cases, after albumin therapy, suggests that whole blood should be given subsequently, when available.

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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

IX. THE TREATMENT OF SHOCK WITH CONCENTRATED HUMAN SERUM ALBUMIN: A PRELIMINARY REPORT [1,2]

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We have had the opportunity to treat 6 patients in shock with concentrated human serum albumin, prepared from pooled normal human plasma by the method of Cohn and co-workers (1).[3] Five of these patients had circulatory failure from hemorrhage and the sixth from extensive burns. These observations were made at the beginning of our experience with the technics utilized in the study of shock by Cournand and co-workers (3). At this time, routine determinations of the cardiac output before and after therapy were not being made.

METHODS

The arterial pressure was recorded from the femoral artery by the method of Hamilton (4). The mean pressure was computed from planimetric measurement of these tracings. The right auricle was catheterized by a method only slightly modified from that reported by Cournand and Ranges (5). Mixed venous blood was obtained from the right auricle and arterial blood from the femoral artery. The oxygen content of these specimens was determined by the method of Van Slyke (6). Oxygen consumption was determined by measurement of the volume and composition of a 2-minute sample of expired air obtained in a Douglas Bag. From these data, the cardiac output was calculated, utilizing the Fick principle. Plasma volume determinations were carried out with the use of the blue dye, T-1824, as described by Gregersen, Gibson, and Stead (7), and adapted to the Klett photoelectric colorimeter or the Beckman quartz spectrophotometer. Plasma protein concentration was determined by the specific gravity method of Barbour and Hamilton (8).

RESULTS

Case 1. A 45-year-old Negro was admitted to Grady Hospital because of stab wounds of the right forearm, forehead, and right flank. He had bled profusely, but there was no evidence of penetration into the peritoneal cavity. He had been drinking whiskey and the blood alcohol concentration was 180 mgm. per cent. The tongue was pale, the radial pulse was weak, and the extremities were cool. There was no sweating. The veins of the forearm were visible and appeared full. The venous blood from the antecubital vein was dark. The systolic brachial blood pressure by the auscultatory method was 80 mm. of Hg, the diastolic, 60 mm. The pulse rate was 96 beats per minute. The observations recorded in Table I were made and at the end of this time the patient's condition seemed unchanged. Two hundred cc. of a 25 per cent solution of albumin were given in 50 minutes. Following this, further studies were made. During the period of time from the beginning of the auricular catheterization to the completion of the second cardiac output, the patient received 200 cc. of normal saline by way of the catheter. The patient improved steadily while the albumin was administered and had no untoward reaction. His hands became warmer, the volume of the pulse increased, and he was less restless. His convalescence was uneventful.

Case 2. A 20-year-old Negress was admitted to the Grady Hospital soon after being stabbed in the posterior aspect of the right chest. The patient was slightly restless; the extremities were cool and somewhat moist. By auscultation, the arterial pressure was 80/60. Physical examination revealed evidence of fluid in the right chest. Preliminary observations were carried out, the results being shown in Table I. Following this, 50 grams of albumin in 200 cc. of fluid were given in 30 minutes. During the infusion, there were no untoward symptoms and the patient became more alert and cooperative. Observations made at this time showed the changes shown in Table I.

Case 3. A 30-year-old Negro came to the Grady Hospital Emergency Clinic a few minutes after being stabbed

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Emory University School of Medicine.

[2] The products of plasma fractionation employed in this work were developed from blood, collected by the American Red Cross, by the Department of Physical Chemistry, Harvard Medical School, Boston, Massachusetts, under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[3] This study was carried out in order to provide more detailed information on the mechanism of the action of albumin in shock than was possible in the original clinical appraisal (2).

TABLE I

Summary of observations on Cases 1 and 2

	Case 1. Male, age 45 years. Stabbed chest with hemothorax. Surface area 1.68 sq. meters		Case 2. Female, age 20 years. Stabbed chest with hemothorax. Surface area 1.46 sq. meters	
	Initial observations	After 50 grams of albumin	Initial observations	After 50 grams of albumin
Femoral aterial pressure (*mm. Hg*)				
systolic	90	110	80	96
diastolic	56	62	48	58
mean	68	81	62	71
Auricular pressure (*mm. water*)	15 to 20	55 to 60		50
Pulse (*beats per minute*)	88	100	68	75
Hematocrit reading	36	31	26	20
Plasma protein (*grams per 100 cc.*)	5.6	5.6	5.6	5.5
Plasma volume (*cc. per square meter*)	1490	1850	1300	1850
Arterial oxygen content (*volumes per 100 cc.*)	14.5	12.6	9.4	7.4
Mixed venous oxygen content (*volumes per 100 cc.*)	10.7	10.0	5.7	4.2
A-V oxygen difference (*volumes per 100 cc.*)	3.8	2.6	3.7	3.2
Oxygen consumption (*cc. per minute per sq. meter*)	116		122	123
Cardiac output (*liters per minute*)	5.1		4.8	5.5
Cardiac index (*liters per minute per sq. meter*)	3.1		3.3	3.8

in the back with a knife. He had bled profusely and on arrival at the hospital the wound was spurting blood. The wound was in the left lower posterior chest and extended subcutaneously into the costovertebral angle, but there was no evidence of hemo- or pneumo-thorax. After closure of the laceration and stoppage of the bleeding, the patient was slightly dull mentally, his skin was moist and cool, the brachial arterial pressure was 150/68 by the auscultatory method, and the pulse rate was 120 per minute.

A plasma volume determination was carried out, but readings were unsatisfactory because of lipemia. The hematocrit reading was 40 per cent. Hamilton manometer tracings taken from the femoral artery at this time showed a pressure of 148/84. The mean pressure was 105 mm. of Hg and the pulse rate, 114. One hundred cc. of a 25 per-cent solution of albumin were rapidly given, intravenously. There was no untoward reaction. Following the albumin solution, the hematocrit reading was 37 per cent. The arterial pressure stabilized at 160/94. The mean pressure was 115 mm. of Hg. The pulse rate was 96 beats per minute. The patient's mental state definitely improved. Over an hour later, 750 cc. of physiological saline solution were given intravenously without change in the patient's condition.

Case 4. A 33-year-old Negro was admitted to the Emergency Clinic about 15 to 20 minutes after receiving a stab wound of the left thigh. It had bled profusely and on admission the patient was confused, his extremities were cold and moist, and the arterial pressure by the auscultatory method was 70/50 mm. of Hg.

The hematocrit reading was 38 per cent and plasma volume, approximately 2400 cc. (surface area 1.8 square meters). Hamilton manometer tracings at this time revealed a femoral pressure of 47/28 and a mean pressure of 37 mm. of Hg. The pulse rate was 99 beats per minute. One hundred cc. of a 25 per cent solution of albumin were given intravenously. The arterial pressure increased to 88/52, and the mean pressure rose to 61 mm. of Hg. The pulse rate was 114 beats per minute. There was no evidence of any untoward reaction. The hematocrit reading fell from the control level of 38 to 32 soon after the albumin infusion was completed, later falling to 30. This represented an increase in plasma volume of 800 cc. One and one-half hours later, 500 cc. of normal saline solution were given intravenously. The hematocrit reading after the infusion was 29 and the arterial pressure had not changed. The patient's temperature remained normal.

Case 5. A 30-year-old Negro male was stabbed in the left temporal region, apparently involving the major temporal vessels. Despite the severe bleeding, it was almost an hour before he reached the hospital. On arrival, he was still bleeding considerably. His blood pressure at this time by the auscultatory method was 90/60, but in general, his condition seemed to be good. Considerable difficulty was encountered in controlling the severed temporal vessels, which had retracted. After about an hour, he attempted to sit up in the Emergency Clinic, but collapsed. It was then noted that his pulse was weaker and more rapid. He failed to improve by further rest in the Trendelenburg position. Blood volume determination about 3½ hours after injury revealed a plasma volume of 3200 cc. (surface area of 2 square meters), and a hematocrit reading of 40. The femoral arterial pressure at this time was 86/54. The mean femoral pressure was 68 mm. of Hg. About 4½ hours after injury, 200 cc. of a 25 per cent albumin solution were given intravenously, in approximately 35 minutes. The femoral arterial pressure rose to 106/60 after the albumin. The mean pressure was 72 mm. of Hg. The patient stated that he felt much better and his level of awareness appeared to rise. There was no evidence of any untoward reaction. The oral temperatures before and after were normal. The hematocrit

TABLE II

Summary of observations on Case 6

Female, age 58. Severe second and third degree burns, previous hypertension (surface area 1.6 sq. meters)

	Initial observations at 12:36 a.m. 1,000 cc. plasma already received	Observations at 2:17 a.m. after 500 cc. of physiological saline solution	Observations at 7:37 a.m. after 3,000 cc. of saline and glucose solutions	Observations at 9:25 a.m. after 50 grams of albumin
Femoral arterial pressure (*mm. Hg*)				
systolic	128 to 190*	128 to 168*	118 to 150*	156 to 204*
diastolic	80 to 100	76 to 92	78 to 88	86 to 106
mean	117	98	95	126
Auricular pressure (*mm. water*)	15 to 20	5 to 10	5 to 10	50 to 55
Pulse (*beats per minute*)	115	94	136	136
Hematocrit reading	39	38	39	33
Plasma protein (*grams per 100 cc.*)	6.4	5.9	5.9	5.5
Arterial oxygen content (*volumes per 100 cc.*)	16.1	15.7	15.6	13.2
Mixed venous oxygen content (*volumes per 100 cc.*)	10.9	9.8	10.5	8.9
A-V oxygen difference (*volumes per 100 cc.*)	5.2	5.9	5.1	4.3
Oxygen consumption (*cc. per minute per sq. meter*)	141	113	112	160
Cardiac output (*liters per minute*)	4.3	3.0	3.5	5.9
Cardiac index (*liters per minute per sq. meter*)	2.7	1.9	2.2	3.7

* Maximum and minimum respiratory variations.

reading fell to 34 per cent, representing a 900 cc. increase in plasma volume.

Case 6. A 58-year-old Negress was admitted to the hospital soon after receiving a severe burn. Seven years previously she had suffered from a hemiplegia and there was a history of high blood pressure. On the night of admission, her dress had become ignited, and on entry to the hospital, it was estimated that about 60 to 70 per cent of the body surface was involved in second and third degree burns. Because of the extent of the involvement, auscultatory blood pressure readings were not obtained on admission, and the peripheral pulses were not palpable. The burns were immediately treated with boric acid ointment dressing and pressure bandages on the extremities. During this time, she received 1000 cc. of plasma. Following this, circulatory studies were carried out and the results are tabulated in Table II. There was a very marked respiratory variation in the arterial pressure, the values recorded being the maximum and minimum values. Because of considerable hemoglobinemia, blood volume determinations were unsatisfactory. Physiological saline solution was allowed to drip through the catheter at the rate of 200 cc. per hour. Almost 2 hours later, another set of determinations was carried out. Following this, 1500 cc. of 5 per cent glucose in distilled H_2O were given in addition to the saline solution dripping through the catheter, and further circulatory measurements were made. Later, 200 cc. of 25 per cent albumin solution were given in 45 minutes. There was no evidence of toxic reaction and, as shown by the data recorded in Table II, the circulation improved greatly. No further therapy was given at this time and the patient's condition remained good, requiring no further plasma for almost 10 hours.

DISCUSSION

The concentrated albumin solutions were easy to administer and caused no unfavorable reactions in this group of patients. In each instance, the patient showed signs of clinical improvement, and where measurements were made, this improvement could be charted quantitatively. In each of the 6 patients, the femoral arterial pressure showed a significant rise. The hematocrit reading uniformly decreased without a corresponding decrease in the protein concentration. In 4 instances, plasma volume determinations were made before treatment. Calculating the increase in the plasma volume from the initial determination and the hematocrit reading before and after receiving the albumin, the plasma volume increased 600, 800, and 900 cc. in the patients receiving 50 grams of albumin. The average increase in volume was 16 cc. for each gram of albumin. This compares closely with observed data of Heyl, Gibson, and Janeway (9), who report that 1 gram of albumin produced an average increase in volume of 17.4 cc. and of Scatchard, Batchelder, and Brown (10), who find 18 cc. per gram by *in vitro* measurements of osmotic pressure. These 3 patients received sufficient albumin to restore the arterial pressure to approximately the normal level. Case 4 was

given only 25 grams of albumin and this was insufficient to raise the arterial pressure to the normal level. One hour after the administration of albumin was completed, the increase in plasma volume measured 800 cc. It is felt that the large increase in plasma volume in this patient was caused in part by the albumin, but in part represented the usual hemodilution by extracellular fluid which occurs when insufficient protein is given to restore the plasma volume to normal.

In the 2 cases in which it was measured, the auricular pressure rose as the plasma volume increased. The difference between the oxygen content of the mixed venous blood and the arterial blood decreased as the circulation improved, and in the 2 cases in which the determination was made, the cardiac output increased. This was particularly striking in Case 6, in whom 3 cardiac output determinations were made before the albumin was given. After the administration of 50 grams of albumin, the cardiac output had increased from 3.5 to 6.4 liters per minute.

SUMMARY AND CONCLUSIONS

A concentrated solution of human serum albumin was given to 6 patients with circulatory failure associated with a decrease in blood volume. In each case, there was distinct clinical improvement without evidence of any undesirable side effects. The increase in plasma volume was commensurate with the predicted osmotic effect of the albumin. Determinations of the arterial pressure, the auricular pressure, and the cardiac output, all revealed the beneficial effects of the albumin.

This work was carried out with the technical assistance of Miss Maurine Giese, Miss Eloise Cavin, and Mrs. Janet Stegeman.

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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

X. THE CONCENTRATIONS OF CERTAIN ANTIBODIES IN GLOBULIN FRACTIONS DERIVED FROM HUMAN BLOOD PLASMA [1,2]

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INTRODUCTION

The separation of the albumin fraction from human blood plasma (1) made available, as by-products, the serum globulins which have been further separated and concentrated. For the sake of convenience, we again present here the list of these fractions, including the albumins, which have been derived from the plasma.

Fraction I	—Largely fibrinogen
Fraction II + III	—Largely β- and γ-globulin
Fraction IV	—Largely α- and β-globulin
Fraction V	—Albumin
Fraction VI	—Small amounts of protein in mother liquors, largely α-globulin and albumin

The so-called Fraction II + III, which contains most of the gamma globulins, has subsequently been broken into three fractions designated II, III-1, and III-2.

Since it is a matter of common knowledge that, in immune sera, antibodies against a variety of pathogenic agents and other antigens are associated with or actually consist of globulins and, since large quantities of globulins from human sera were made available as by-products in the preparation of albumin, it became of interest, both from a theoretical and a practical standpoint, to determine the concentration of various antibodies in these globulin fractions. Thus, for example, to the immunologist, precise knowledge would be of great value as to whether normal antibodies in human serum were all confined to a single species of globulin or whether those reacting with certain antigens (*e.g.*, proteins) were represented by one globulin and those with other antigens (*e.g.*, polysaccharide-protein complexes) by one or more different globulins. Furthermore, from the theoretical standpoint, it would be of interest to know whether the so-called normal antibodies of human origin in their chemical behavior closely resemble or are identical with immune antibody or whether they exhibit quite different properties. On the practical side, it is obvious that if antibodies directed against certain pathogenic agents were found to be present in sufficient concentration in one of the globulin fractions mentioned above, the material might have an immediate application to the prevention or therapy of the corresponding disease.

[1] This work has been carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[2] This paper is Number 18 in the series "Studies on Plasma Proteins" from the Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross.

[3] The large number of the investigators who have carried out tests for certain antibodies rendered it impracticable to include as co-authors all the participants in the work to be described in this paper. Accordingly, grateful acknowledgment is here made for their insight and advice to Dr. Elliott Robinson of the Massachusetts Antitoxin and Vaccine Laboratory and Dr. A. R. Dochez of the Committee on Medical Research of the Office of Scientific Research and Development and to the following individuals for their share in providing certain of the data presented in this communication: Dr. W. C. Boyd, Boston University, Dr. W. L. Bradford, University of Rochester, Dr. M. D. Eaton, California Department of Public Health, Dr. G. Edsall, Massachusetts Antitoxin and Vaccine Laboratory, Dr. I. C. Hall, University of Colorado, Dr. G. K. Hirst, Rockefeller Institute, Dr. C. A. Janeway, Children's Hospital, Boston, Dr. S. D. Kramer, Michigan Department of Health, Dr. S. Mudd, University of Pennsylvania, Dr. M. F. Shaffer, Formerly of the Mass. Antitoxin and Vaccine Laboratory, Dr. J. Stokes, Jr., Children's Hospital, Philadelphia, and Dr. A. Wadsworth of the State of New York Health Department.

With these considerations in mind and with the collaboration of a number of investigators, an analysis for the following antigens of the antibody content of the various globulin fractions was undertaken.

Agglutinins against typhoid O antigen
Agglutinins against typhoid H antigen
Agglutinins against B. pertussis, phase I
Complement fixing antibody against influenza A virus, strain PR8
Complement fixing antibody against the virus of mumps
Inhibitor of the hemoagglutinin of influenza A virus
Mouse protective (neutralizing) antibody against influenza A virus
Mouse protective (neutralizing) antibody against swine influenza virus
Mouse protective (neutralizing) antibody against virus of poliomyelitis
Diphtheria antitoxin
Streptococcal antitoxin
Isoantibodies against cells of human blood groups A and B [4]

Tests for all of these antibodies were not carried out with specimens of all the fractions when it was found that certain of them, as will be subsequently shown, contain little or no antibody of any sort. The majority of determinations of antibody concentration have been confined to a large number of preparations of Fraction II + III and Fraction II. Even with these fractions, however, only a few tests have been made for the presence of certain of the antibodies mentioned above. However, tests for the antibody content of Fraction II, corresponding to 6 representative antigens, *i.e.*, the typhoid antigens, mumps virus, influenza A virus, diphtheria and streptococcal antitoxin, have been carried out as routine on a large number of preparations with the purpose of establishing a preliminary standard of potency. Accordingly, in the description of the technics employed in making these tests, which are given below, those procedures which have been invoked only occasionally are not included.

[4] As soon as it was determined that these antibodies were separated in Fraction II+III, their further investigation was undertaken first by Dr. W. C. Boyd and later by Lt. L. Pillemer, J. L. Oncley, M. Melin, Capt. J. Elliott and Lt. M. C. Hutchinson. Their results are considered in a separate paper in this series.

METHODS

We present the following description of the methods which have been used in the laboratory of the Department of Bacteriology and Immunology at the Harvard Medical School in testing for certain of the antibodies. In addition is included reference to the procedure adopted by Doctors A. Wadsworth and M. Kirkbride and Miss J. Hendry, at the State of New York Department of Health Laboratories, for determining the streptococcal antitoxin content of the fractions.

Determination of H and O agglutinins for E. typhosa

The fraction (or plasma) is diluted in saline from 1 to 4 to 1 to 256 by decrements of twofold, employing volumes of 2 ml. each.

H agglutinins. To 0.5 ml. of each dilution is added 0.5 ml. of a suspension of *E. typhosa* containing the H antigen.[5] As a control for the antigen, 0.5 ml. of saline is added to 0.5 ml. of the bacterial suspension. As additional controls, 0.5 ml. of saline is added to 0.5 ml. of each dilution of the fraction, since certain preparations of globulin when diluted may yield precipitates of protein resembling the H but not the O type of agglutination. The mixtures are incubated in the water bath at 48° to 52° C. for 2 hours, allowed to stand at 4° C. for 1 hour, and the degree of agglutination recorded. The dilution of the fraction (or plasma) causing a degree of agglutination denoted by 2+ is taken as the end-point (complete agglutination as shown by the presence of a clear supernate is denoted as 4+).

O agglutinins. To 0.5 ml. of each dilution is added 0.5 ml. of a suspension of *E. typhosa* containing the O antigen.[6] As a control for the antigen, 0.5 ml. of saline is added to 0.5 ml. of the bacterial suspension. The mixtures are incubated overnight (16 to 18 hours) at 48° to 52° C. and readings are made the following morning, after the tubes have stood for at least 1 hour at 4° C. The dilution of the fraction causing a degree of agglutination denoted by 2+ is taken as the end-point.

Titration of the substance inhibiting the agglutination of red blood cells by influenza A virus (2)

The fraction is diluted in saline from 1 to 8 to 1 to 256 by decrements of twofold. To 0.5 ml. of each dilution of the fraction are added 0.5 ml. of diluted virus [7] and 1 ml.

[5] Prepared according to the method described in Diagnostic Procedures and Reagents. Am. Public Health Assn., 1st Ed., N. Y., 1941, pp. 236–238.

[6] See footnote 5.

[7] The virus employed is the PR8 strain of influenza A. The chorioallantoic fluid of the developing hen's egg inoculated on the 10th day of incubation is collected on the 12th day and stored at −76° C. in a CO_2 ice cabinet until required. The virus dilution used is 4 times that required to cause 2+ agglutination of a 2 per cent suspension of chicken's red blood cells, according to the standards described by Hirst as indicated above.

of 2 per cent chicken red blood cells. The latter are washed four times using about 50 ml. of saline for each washing. The mixtures are allowed to stand at room temperature together with standards of comparison prepared in the following manner.

A	1 per cent red blood cells	=1 ml. 2 per cent susp. +1 ml. saline
B	0.75 per cent red blood cells	=0.75 ml. 2 per cent susp. +1.25 ml. saline
C	0.5 per cent red blood cells	=0.5 ml. 2 per cent susp. +1.5 ml. saline
D	0.3 per cent red blood cells	=0.3 ml. 2 per cent susp. +1.7 ml. saline

Readings are made after 1 hour by comparison with these standards:

Mixtures with a density of the supernatant equivalent to A = O
Mixtures with a density falling between A + B = 1+
Mixtures with a density falling between B + C = 2+
Mixtures with a density falling between C + D = 3+
Mixtures with a density less than D = 4+

We have taken the O reading as the end-point since we believe it is the most easily and accurately determined, although Hirst has employed the 2+ end-point. All end-points are expressed in terms of final dilutions of fraction or plasma in the mixture.

Complement fixation test for mumps and influenza A

Removal of anticomplementary activity of Fraction II. Since nearly all the globulin fractions have exhibited a greater or less degree of anticomplementary activity, in most of our work, they have been treated with trypsin which has been found frequently to remove this property without markedly reducing the concentration of antibody. "Bacto" trypsin has been employed, but since each lot has a different strength, it is necessary to run titrations to determine the dilution of trypsin that must be used. Most of the tests in which trypsinization has been employed have been carried out with one lot of trypsin which has been used in a quantity of 0.05 ml. of undiluted trypsin added to 1 ml. of the globulin fraction, so diluted in saline as to contain 4 per cent protein. The mixtures are incubated in the water bath at 37° C. for 30 minutes, then heated at 60° C. for 20 minutes. They are then diluted with saline *immediately*, using a volume of 1 ml., from 1 to 20 to 1 to 640 by decrements of two. The results, obtained by this method, both with influenza A and mumps antigen, cannot be accepted as accurately quantitative since the precise degree of antibody inactivation due to trypsinization cannot be precisely determined. The method has also not infrequently proved unsatisfactory, since even when anticomplementary activity is eliminated by trypsinization, certain globulin fractions fix complement in the presence of normal egg or parotid gland material.

Recently, a method of adding sufficient complement to satisfy the anticomplementary activity of Fractions II + III and II has been devised which has proved more satisfactory than that of trypsinization. The number of units of complement fixed by a 1 to 20 dilution of the fraction is determined by setting up a series of 10 tubes, each containing 0.125 ml. of a 1 to 20 dilution of the fraction. Complement is then titrated in the usual manner (see below) to determine the number of ml. of 1 to 60 dilution which contain 1 unit. The volume of a 1 to 10 dilution of complement which contains 1 unit is then calculated. Various amounts of 1 to 10 dilution of complement, containing from 1 to 10 units, are then added to each tube containing the 1 to 20 dilution of fraction.

Example

0.125 ml. of 1 to 20 fraction +1 unit comp. (0.02 ml. of 1 to 10)
0.125 ml. of 1 to 20 fraction +2 units comp. (0.04 ml. of 1 to 10)
0.125 ml. of 1 to 20 fraction +3 units comp. (0.06 ml. of 1 to 10)
0.125 ml. of 1 to 20 fraction +4 units comp. (0.08 ml. of 1 to 10)

0.125 ml. of 1 to 20 fraction +10 units comp. (0.20 ml. of 1 to 10)
Comp. titer: 0.12 ml. of 1 to 60 = 1 unit, therefore 0.02 ml. of 1 to 10 = 1 unit.

The volumes are adjusted with saline to equal the volume of the 10th tube. These mixtures are allowed to stand overnight at 4° C. and 0.25 ml. of sensitized cells are added the next morning. They are incubated for ½ hour at 37° C. in the water-bath and read. The end-point is taken as the number of units of complement that are almost completely fixed by the fraction.

Example

	Units of complement									
	1	2	3	4	5	6	7	8	9	10
0.125 ml. of a 1 to 20 dil. of fraction A66	4+	4+	4+	3+	3+	1+	tr.	0	0	0
0.125 ml. of 1 to 20 dil. of fraction MP 1,2,3,A	4+	4+	4+	3½+	3+	tr.	0	0	0	0

In the example given above, 0.125 ml. of 1 to 20 dilution of both fractions fixes about 5 units of complement. By titration, it was determined that 1 unit of complement was contained in 0.12 ml. of 1 to 60. Therefore, 3 ml. of 1 to 20 fraction fixed about 120 units of complement (24 × 5 units) or 14.4 ml. (120 × 0.12 ml.) of 1 to 60 dilution which was equivalent to 0.24 ml. of undiluted complement. Therefore, 0.24 ml. of undiluted complement was added to 3 ml. of 1 to 20 dilution of each of the fractions.

The mixtures were allowed to stand overnight at 4° C. and then heated at 55° C. for 15 minutes to inactivate any slight excess of complement. Dilutions were then prepared and the complement fixation test carried out as described below. The results were as follows:

Example

Fr. II MP 1, 2, 3, A

	Initial dilutions of fraction						
	1 to 20	1 to 40	1 to 80	1 to 160	1 to 320	1 to 640	1 to 1280
MP (mumps parotid susp.)	4	4	4	4	1	0	0
NP (normal parotid susp.)	0	0	0	0	0	0	0
PR8 (virus in egg fluid)	4	3	1	0	0	0	0
Normal egg (fluid)	0	0	0	0	0	0	0
Comp. control	sl. tr.	0	0	0	0	0	0
Hem. control	4	4	4	4	4	4	4

Fr. II-A66

MP	3	1	tr.	0	0	0	0
NP	0	0	0	0	0	0	0
PR8	3	3	2	tr.	0	0	0
N. egg	0	0	0	0	0	0	0
Comp. cont.	0	0	0	0	0	0	0
Hem. cont.	4	4	4	4	4	4	4

4 = complete absence of hemolysis

Plasma. Pooled plasmas, from which the fractions are derived, have shown little anticomplementary activity. Accordingly, they are heated at 60° C. for 20 minutes and then tested in the usual manner. If they do exhibit some anticomplementary activity, they are heated for a second time at 60° C. for 20 minutes after they are diluted. This procedure has been shown not to reduce significantly the antibody content but is highly effective in removing the anticomplementary effect of plasma or serum but not that of the fractions.

Hemolytic system. To one volume of a 2 per cent suspension of thoroughly washed sheep's red blood cells, one volume of diluted anti-sheep red cell rabbit serum, containing 2 units of amboceptor, is added, 15 minutes before the sensitized cells are used in the test.

Complement. Sera from at least 6 guinea pigs are collected, pooled, and stored in the CO_2 cabinet (−76° C.). No diminution in complement titer has been observed under these conditions during a period of about 2 months. Titrations of complement, however, are performed each time the complement fixation test is done. Two titrations are carried out simultaneously, in one of which the volumes of complement are twice those employed in the other. This is done as a check on the titration utilizing the smaller volumes, since errors may be easily introduced in the latter due to the necessity of measuring such small quantities. The unit of complement is taken as the smallest amount that gives complete hemolysis in the titration which includes the smaller volumes.

Double volume titration. Complement is diluted 1 to 60. Of this dilution, 0.40 ml., 0.38 ml., 0.36 ml., 0.34 ml., 0.32 ml., 0.30 ml., 0.28 ml., 0.26 ml., 0.24 ml., and 0.22 ml. are pipetted into a series of tubes. These volumes are then brought up to 1 ml. with saline and 0.5 ml. of sensitized cells are added.

Standard complement titration. Complement is diluted 1 to 60. Of this dilution, 0.20 ml., 0.19 ml., 0.18 ml., 0.17 ml., 0.16 ml., 0.15 ml., 0.14 ml., 0.13 ml., 0.12 ml., and 0.11 ml. are pipetted into a series of tubes. These volumes are then brought up to 0.5 ml. with saline and 0.25 ml. sensitized cells are added. The contents of the tubes are mixed and incubated in the water bath at 37° C. for 30 minutes. For use in the test, the complement is diluted so that 0.15 ml. contains 2 units.

Antigens

Mumps parotid. One volume of a 20 per cent emulsion of infected monkey parotid [4] is diluted with 5 volumes of saline and rotated for 30 minutes at 3500 r.p.m. in an angle centrifuge. The supernatant fluid is then titrated with 3 known strongly positive mumps convalescent sera (monkey or man), in dilutions of 1 to 8. Three known mumps negative sera (monkey or man), diluted 1 to 8, are included as controls. For use in the complement fixation test, twice the concentration of antigen giving complete fixation (4+) in this titration is employed.

Normal parotid. Parotid gland from a normal monkey is prepared in the same manner as that employed in the case of the infected gland and used in a dilution equivalent to that of the mumps parotid.

Influenza A antigen. The PR8 strain of influenza A is used as antigen. The chorioallantoic fluid of the developing hen's egg, inoculated on the 10th day of incubation, is collected on the 12th day and stored at −76° C. in a CO_2 cabinet until required. It should be of such a strength as to give 2+ agglutination with 2 per cent hen cells in final dilution of 1 to 128 in the Hirst test. In the complement fixation test, the chorioallantoic fluid is employed in a dilution of 1 to 8.

Normal egg. Chorioallantoic fluid is collected from embryonated hen's eggs on the 10th to 12th day of incubation at 102° to 103° F. and stored at −76° C. in a CO_2 cabinet until required, when it is used in a dilution equivalent to that of the influenza A antigen.

The complement fixation test. To 0.125 ml. of each dilution of the fraction, 2 units of complement in a volume of 0.15 ml. and 0.1 ml. of antigen or corresponding control material are added.

Six sets of dilutions of the fraction in saline are prepared. To one set, mumps parotid suspension is added. To the second, normal monkey parotid is added, to the third row, the influenza A antigen, and to the fourth row, normal chorioallantoic fluid are added. The fifth row receives saline instead of any of these reagents, and thus represents a control on the anticomplementary activity of each dilution of the fraction. The sixth row receives neither complement nor antigen but contains only 0.125 ml. of each dilution of fraction and saline thus serving as hemolytic control.

The mixtures are placed overnight in the ice box at 4° C. and 0.25 ml. of sensitized sheep's red blood cells are added to each on the following morning. They are incubated ½ hour at 37° C. in the water-bath and read. The end-point is taken as the last dilution of the fraction (initial dilution) giving definite fixation of complement which is denoted as 2+. The following controls are included in which the volumes are all rendered equivalent to those employed in the test by the addition of the appropriate quantity of saline.

Mumps parotid gland + 2 units of complement
Normal parotid + 2 units of complement
PR8 egg fluid + 2 units of complement
Normal egg fluid + 2 units of complement
2 units complement
1 unit complement
Saline
Known + serum (mumps)
Known − serum (mumps)
Fraction A66 (standard for influenza antibody)

Titration of anticomplementary activity

The fraction is diluted in saline by decrements of 2 (1 to 4 to 1 to 256), employing volumes of 0.5 ml. With these dilutions, the test is carried out as follows. To 0.125 ml. of each dilution of the fraction, 2 units of complement prepared in the manner described above are added in a

[4] Enders, J. F., and Cohen, S., Proc. Soc. Exp. Biol. and Med., 1942, 50, 180-184.

volume of 0.15 ml. To adjust the total volume of fluid to that employed in the complement fixation test, 0.1 ml. of saline is added.

The mixtures are allowed to stand overnight at about 4° C., when to each is added 0.25 ml. of a 2 per cent suspension of sensitized sheep's red blood cells. The tubes are then placed in a water bath at 37° C. for ½ hour and the fixation of complement then recorded as 0, tr., 1+, 2+, 3+, and 4+. Complete absence of hemolysis is denoted by a reading of 4+. The end-point of anticomplementary activity is indicated by that dilution (initial dilution) of the fraction showing a residue of non-hemolyzed cells read as "tr."

Mouse test for influenza A protective (neutralizing) antibody

The fractions are usually diluted in sterile infusion broth to 1 to 40, 1 to 80, 1 to 160, 1 to 320. Plasmas from which fractions have been prepared are diluted in broth to 1 to 2, 1 to 4, 1 to 8, 1 to 16. In preparing mixtures of virus and solution, either fraction or plasma, 0.5 ml. of dilution is mixed with 0.5 ml. of the virus [9] using *a clean pipette for each dilution.*

The mixtures are allowed to stand at room temperature for 20 minutes. All series of mixtures not being employed for inoculation are maintained at 4° C. while the mice are receiving injections. Under light ether anesthesia, 4 mice are each inoculated intranasally with 0.05 ml. of each mixture. The virulence of the virus is determined in each experiment, employing dilutions of 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}, prepared in infusion broth.

Specific deaths, confirmed by autopsy, are recorded over a period of 10 days. The 50 per cent mortality end-point is calculated according to the method of Reed and Muench (3).

Rabbit skin test for diphtheria antitoxin

Dilution of standard antitoxin. Diphtheria antitoxin standardized to contain 6 u in 1 ml. is employed. A key dilution of 1 to 30 is made and will be referred to as (A). According to the following procedure, dilutions of the standard antitoxin, ranging from 0.08 to 0.14 units of antitoxin per ml., are then prepared from the key dilution.

2.8 ml. (A) + 1.2 saline = 0.14 u/ml.
2.6 ml. (A) + 1.4 saline = 0.13 u/ml.
2.4 ml. (A) + 1.6 saline = 0.12 u/ml.
2.2 ml. (A) + 1.8 saline = 0.11 u/ml.
2.0 ml. (A) + 2.0 saline = 0.10 u/ml.
1.8 ml. (A) + 2.2 saline = 0.09 u/ml.
1.6 ml. (A) + 2.4 saline = 0.08 u/ml.

This series of dilutions is designated the L+/12 standard and is employed in determining the antitoxic potency of the fractions. An L+/120 standard prepared in a similar manner is used with the plasmas which includes an antitoxin range from 0.008 to 0.014 u/ml.

Dilutions of fractions. The fractions are diluted wit normal saline so as to contain the following amounts of th fraction in 1 ml.: 0.008 ml., 0.01 ml., 0.015 ml., 0.020 ml 0.025 ml., 0.029 ml., 0.04 ml., 0.05 ml., 0.075 ml., 0.10 ml 0.15 ml., and 0.20 ml. It is usually possible to find th end-point within these ranges, but occasionally a narrowe range may be required.

Toxin. For the titration of the fractions, the diphtheri toxin is diluted to contain 1/12 L+ dose per ml. Fo plasmas, it is diluted so that 1/120 L+ is contained in 1 m It is rather unstable and should be mixed without bubblin just before using.

Procedure. To a series of tubes, each of which contair 1 ml. of the toxin dilution, are added 1 ml. each of th fraction or standard antitoxin dilutions. The toxin an fraction dilution or toxin and antitoxin dilution is mixe by inverting the tube three times. Of each mixture, 0. ml. is injected intradermally into the skin of a New Zealan white rabbit. Two rabbits should be injected with sample of the same mixtures in order to provide a check and, i the results do not agree, the test should be repeated. Th reactions are read from 66 to 72 hours after injection.

Calculation of end-points. On each rabbit, the first in jection site in each series which shows no reaction ("wipe out") is recorded and compared with effect of the standar antitoxin.

Example. The smallest quantity of fraction which neu tralized the test dose of toxin was found t be 0.029 ml. In the same experiment, th least amount of the standard antitoxin which neutralized the test dose of toxin was found to be 0.1 unit.
Let X = number of units of antitoxin per 1 ml. of the fraction, then $0.029:0.1::1.0:X$
$X = 3.4$ units.

Determination of streptococcal antitoxin

The procedures employed in determining the content of streptococcal antitoxin of fractions and plasmas were in most instances those already published (4) and need not therefore be described in this place. These tests were carried out by Miss Jessie L. Hendry of the State of New York Department of Health. She has occasionally modified the technique. Thus, material of low antitoxic potency is tested by employing one skin test dose of toxin as the test dose instead of the standard 5 skin test doses.

RESULTS

The association of antibody with Fraction II + III

Much evidence is available which indicates that antibody consists of modified globulin and is usually, although not exclusively, associated with the gamma globulins. It was expected, therefore, that most of the antibody in human plasma would be found in Fraction II + III, since this material contains about 90 per cent of

[9] The virus employed is the PR8 strain of influenza A propagated in the developing chicken embryo as described in footnote 7 and is usually diluted so that 0.05 ml. contains about 3000 50 per cent M. D.

TABLE I

Antibodies in globulin Fraction II + III derived from human plasma [a]

Antibody	Type of antibody	Investigator	Institution	Concentration comp. to plasma
Anti-diphtheria	Antitoxin neutralizing	Edsall	Mass. Antitoxin and Vaccine Laboratory	10
Anti-dysentery	Agglutinins	Mudd	Univ. of Pennsylvania	2–10
Anti-herpes simplex	Neutralizing	Stokes	Children's Hosp., Phila.	*
Anti-influenza (human PR8)	Hirst inhibition	Hirst	Rockefeller Institute	4–8
Anti-influenza (human PR8)	Hirst inhibition	Eaton	Calif. Dept. Public Health	4
Anti-influenza (human PR8)	Hirst inhibition	Enders	Harvard Med. School	10–15
Anti-influenza (human PR8)	Complement fixation	Enders	Harvard Med. School	10–15
Anti-influenza (human PR8)	Neutralizing	Stokes	Children's Hosp., Phila.	10
Anti-influenza (human PR8)	Neutralizing	Enders	Harvard Med. School	9
Anti-influenza (swine)	Neutralizing	Stokes	Children's Hosp., Phila.	10
Anti-influenza (swine)	Neutralizing	Shaffer	Mass. Antitoxin and Vaccine Laboratory	4
Anti-lymphocytic choriomeningitis	Neutralizing	Stokes	Children's Hosp., Phila.	*
Anti-measles	Protective (human)	Stokes	Children's Hosp., Phila.	
Anti-mumps	Complement fixation	Enders	Harvard Med. School	2–10
Anti-parapertussis	Agglutinins	Mudd	Univ. of Pennsylvania	64
Anti-pertussis	Agglutinins	Mudd	Univ. of Pennsylvania	4–10
Anti-pertussis	Agglutination	Enders	Harvard Med. School	10
Anti-pertussis	Mouse protection	Bradford	Univ. of Rochester	4–10
Anti-perfringens	Protective	Hall	Univ. of Colorado	*
Anti-poliomyelitis	Neutralizing	Kramer	Mich. Dept. Health	10
Anti-poliomyelitis	Neutralizing	Stokes	Children's Hosp., Phila.	16
Anti-poliomyelitis	Rat and mice protection	Kramer	Mich. Dept. Health	10
Anti-poliomyelitis	Rat and mice protection	Stokes	Children's Hosp., Phila.	10
Anti-scarlatina	Neutralizing	Bradford	Univ. of Rochester	*
Anti-scarlatina	Neutralizing	Wadsworth	New York Dept. Health	5–10
Anti-streptococcus	Antitoxin	Wadsworth	New York Dept. Health	4–10
Anti-typhoid	H agglutinin	Enders	Harvard Med. School	8–10
Anti-typhoid	O agglutinin	Enders	Harvard Med. School	8–10
Anti-vaccinia	Neutralizing	Janeway	Children's Hosp., Boston	*
Isoagglutinins	Agglutinins	Boyd	Harvard Med. School	8–10

[a] These assays were undertaken at the request of Dr. A. R. Dochez of the Committee on Medical Research of the Office of Scientific Research and Development who wrote to the investigators listed in March, 1942, "In the process of preparing human plasma used for transfusion purposes in the armed forces of the United States, by Dr. Edwin Cohn of Harvard University, a number of fractions of the original plasma result. Only one of these, the albumin fraction, is used for transfusion. It is the desire of the Government to ascertain to what useful purpose the remaining fractions can be put. Among these fractions is one containing the α-, β-, and γ-globulins. As you doubtless know, this fraction contains whatever immune bodies may have been present in the original plasma. In the process of purification approximately ten times concentration of the immune body fraction is effected. It is hoped that these immune bodies may be used practically either for the prophylaxis or treatment of certain infectious diseases. In order to test the validity of such a procedure it is first necessary to titrate the globulin fraction for its content of specific antibodies. . . . The first titrations would be with mixtures of the α-, β-, and γ-globulins. Later fractionation of the different globulins will be performed and the specific immune body containing globulin will be furnished for a similar titration." We are greatly indebted to Dr. W. C. Boyd for compiling this table.

* Activity present but no quantitative data.

this species of protein originally included in the plasma.

In substantiation of this expectation, a considerable number of determinations of the antibody concentration of an 11 to 12 per cent solution of Fraction II + III and of the plasmas from which the various preparations in question were derived (Table I) have shown that in the majority of instances the yield of antibody in Fraction II + III was of the order to be expected if most of the antibody of the plasma were associated with the globulins recovered in this fraction, since the concentration of the latter over the plasma is 8 to 10 times.

Further indication of the almost complete association of antibody with Fraction II + III was

TABLE II

Antibodies in certain fractions of human plasma exclusive of Fraction II + III

Fraction	Antibody	Type of antibody	Investigator	Institution	Concentration comp. to plasma
I	Anti-typhoid	O agglutinin	Enders	Harvard Med. School	<1
	Anti-typhoid	H agglutinin	Enders	Harvard Med. School	1
	Anti-mumps	Complement fixation	Enders	Harvard Med. School	<1
	Anti-influenza (human PR8)	Complement fixation	Enders	Harvard Med. School	<1
	Anti-influenza (human PR8)	Neutralizing	Enders	Harvard Med. School	<1
	Anti-diphtheria	Antitoxin	Enders	Harvard Med. School	<1
IV	Anti-typhoid	O agglutinin	Enders	Harvard Med. School	2
	Anti-typhoid	H agglutinin	Enders	Harvard Med. School	<1
	Anti-influenza (human PR8)	Complement fixation	Enders	Harvard Med. School	<1
	Anti-diphtheria	Antitoxin	Edsall	Mass. Antitoxin and Vaccine Laboratory	1
	Anti-dysentery	Agglutinins	Mudd	University of Pennsylvania	1
	Isoagglutinins	Agglutinins	Boyd	Harvard Med. School	<1
Supernatant of II + III	Anti-typhoid	O agglutinin	Enders	Harvard Med. School	<1
	Anti-typhoid	H agglutinin	Enders	Harvard Med. School	1 or <1
	Anti-mumps	Complement fixation	Enders	Harvard Med. School	<1
	Anti-influenza (human PR8)	Complement fixation	Enders	Harvard Med. School	<1
	Anti-influenza (human PR8)	Neutralizing	Enders	Harvard Med. School	<1
	Anti-diphtheria	Antitoxin	Enders	Harvard Med. School	<1

obtained by testing a few specimens of Fractions I and IV and the supernatant fluids from Fraction II + III. These results are summarized in Table II. From them, it is clear that only small quantities of antibody have been found therein. The failure to detect significant quantities of antibody in the supernatant fluids of Fraction II + III is of especial significance, since it precludes the possibility that any considerable quantity of active material is carried over into Fractions IV, V, and VI.

We may conclude, then, that a very large proportion (it is impossible to give a precise estimate on the basis of the available data) of the various antibodies mentioned in the tables, and present in so-called normal human plasma, can be recovered in Fraction II + III. It cannot be assumed, however, from the evidence so far presented that the antibodies are associated only with the γ-globulin of Fraction II + III, since it will be recalled (1) that the fraction is comprised of about 12 per cent α- and 42 per cent β-globulin in addition to the γ-globulin which represents about 90 per cent of the total plasma γ-globulin. Upon the separation and immunologic analysis of Fraction II, some recent preparations of which contain 99 or more per cent γ-globulin, results have been obtained which strongly suggest the close association of certain antibodies with γ-globulin. The remainder of the experimental portion of this communication will be concerned mainly with a description of our analyses of the antibody content of Fraction II.

The antibodies of Fraction II

Fraction II + III was split into 3 subfractions (1,5) with the objective of concentrating and recovering (*1*) the thrombin in Fraction III-2 as a hemostatic agent, (*2*) the isohemagglutinins in Fraction III-1 for blood typing, and (*3*) a Fraction II containing practically nothing but γ-globulin. This chemical development has resulted in a further concentration in antibody activity. Moreover, with the elimination of α- and β-globulins, the purified γ-globulin exhibits greater stability. Finally, information concerning the association of antibody with the various species of globulin comprising Fraction II + III has resulted from the chemical fractionation of the γ-fraction from the α and β components.

A series of preparations have been prepared in the Plasma Fractionation Laboratory from Fraction II + III by slight alterations in the general method, all designated as Fraction II. These have been prepared and tested by immunologic technics. Those prepared first contained about

80 per cent γ-globulin, whereas the most recent products consist of 96 to 100 per cent γ-globulin.

Selection of a standard of comparison. Early in the course of this work one of these preparations, A66, was selected as a provisional standard and its antibody content determined at the same time as that of each newly prepared specimen of Fraction II. In this way, a considerable quantity of data (Table III) has been accumulated in respect to the standard which serves to indicate the range of variability one may expect when antibody concentrations of Fraction II are determined by the methods we have employed. In certain instances, titrations of the plasma from which A66 was derived have also been carried out simultaneously with those of the fraction itself. In Table IV are presented the results obtained in titrations of the plasma for 5 representative antibodies, *i.e.*, neutralizing antibody for influenza A, inhibitor of influenzal hemoagglutinin, diphtheria antitoxin, and H and O typhoid agglutinins. The plasma was preserved at the Harvard Plasma Fractionation Laboratory in the frozen and dried state. These figures give a conception of the variation of successive determinations for plasma which is, of course, very low in antibody content.

TABLE III

Determination of certain antibodies in Fraction A66 at various times

Determination	Titer of solution						Units diphtheria antitoxin per ml. solution
	Typhoid agglutinin		Mumps comp. fix.	Influenza A			
	O	H		Comp. fix.	Hirst test	Mouse prot.	
1	8	200			512	160	3.3
2	6	128			256		
3	4	256			512	106	3.9
4	6	200			256	106	3.9
5		128			256	160	3.4
6	12	250			256	100	3.8
7	8	200			256	160	3.3
8	8	256			256	120	3.8
9	8	256			256	130	3.5
10	12	200			512	250	3.4
11	12	200	80	320	128	200	3.8
12	8	200	80	320	256	140	3.8
13	6	200					4.2
14	16	64	80	320	256	80	4.0
15	16	64	80	160	256	160	3.8
Average	9.3	187	80*	280*	302	144	3.7

* Average of determinations 11, 12, 14, and 15 only, done by new method for removing anticomplementary activity.

The results of all titrations, with the exception of those for diphtheria antitoxin, are expressed as reciprocals of the dilution of the fraction. All dilutions are given as initial dilutions except those for hemoagglutinin inhibitor (Hirst test) which are final. The diphtheria antitoxin content of the fraction is given in units per ml. The various determinations were carried out during a period of 8 months and were fairly uniformly distributed during this period.

TABLE IV

Determination of influenza A neutralizing antibody, diphtheria antitoxin and typhoid agglutinins in plasma 65–66 at various times

Determination	Hemo-agglutinin inhibition (Hirst test)	Neutralizing antibody Influenza A	Diphtheria antitoxin	Typhoid agglutinin	
				O	H
1	32	5	0.08	4	8
2	32	5	0.07	4	8
3	32	3	0.10	4	23
4	32	3	0.08	4	23
5	32	4	0.11	<4	8
6	32	4	0.12	4	<4
7	32	5	0.12	4	8
8		2.9	0.13		8
9		3.2	0.10		
10		2.5	0.14		
11		10			
12		12			
13		5.3			
14		3.2			
15		5.8			
Average	32	5	0.11	4	11

End-points of O and H agglutinins are expressed as reciprocals of the plasma dilution. The titers of neutralizing antibody are expressed as the reciprocal of the plasma dilution which protected 50 per cent of the mice against the test dose of virus. The diphtheria antitoxin content is expressed in units per ml. of plasma. The determinations were carried out over a period of 7 months. The distribution of the tests over this period was fairly uniform.

An examination of the data in Tables III and IV leads to several conclusions. First, the content of diphtheria antitoxin in both Fraction II and the plasma can be estimated in titrations done at different times with considerably greater accuracy than any of the other antibodies. Second, the rather extensive range in the values for the other antibodies, in particular the influenzal neutralizing substance, makes abundantly clear the necessity for including a standard of comparison, as we have done, in the assay of the

potency of each new and unknown product. In the third place, and most significantly for the purpose of obtaining a high concentration of antibody, a comparison of the average titers of fraction and plasma indicates that with most of the antibodies a concentration much greater than that of Fraction II + III has been attained. The data for diphtheria antitoxin and neutralizing antibody reveal a concentration factor of 34 for the former and 29 for the latter. The factor in the case of hemoagglutinin inhibitor (Hirst test) is lower (about 10). This probably is due to the presence of a non-specific inhibiting substance known to be present in normal plasma (2) which renders the plasma values higher than they should be. The average value for H agglutinin indicates a concentration of this antibody of about 17 times, in contrast to the O agglutinin which at best has been concentrated twofold. In Table IV, we have not included figures for the titrations of the remaining antibodies mentioned in Table III, since only one or two determinations were made. It may be stated, however, that on the basis of the most recent and most trustworthy data, complement fixing antibody for mumps antigen appeared to be concentrated about 20 times and complement fixing antibody for influenza A about 18 times.

In contrast to the large quantities of all other antibodies for which tests were made, those specific for the so-called O or somatic antigen of the typhoid bacillus were present only in low concentration. This observation is of considerable interest since it distinctly points to a qualitatively different antibody in respect to the species of protein of which it is composed. Indeed, if the fact determined by Jones (6) be recalled, *viz.*, that the antibody reacting with the H antigen of the hog-cholera bacillus is definitely more thermostabile than the O antibody, perhaps our finding may not be unexpected.

A few tests for the presence of isoagglutinins in A66 reveal concentration factors of 2 to 4 which are distinctly lower than the values found for Fraction II + III and suggest that the isoagglutinins either may be a different sort of γ-globulin or possibly included in the α- or β-globulin, along with the O antibody. This hypothesis is given additional support by the investigations of Pillemer, Oncley, Melin, Elliott and Hutchinson (7), who have determined conditions for the concentration of most of the isoagglutinin in a fraction corresponding to Fraction III-1.

Immunologic assay of various preparations of Fraction II. In Table V are summarized the various antibody titers obtained with 61 preparations of Fraction II. A brief survey of these data leaves the impression that the products prepared in slightly different ways and tested at rather widely different times are in general fairly uniform in respect to antibody content.

With a few exceptions, the quantity of diphtherial antitoxin is around 2 units per ml. of solution. It is apparent, in view of this fact, that the antitoxin of the standard (3.9 units) is exceptionally high. In the evaluation of the results of diphtheria antitoxin titrations, it should be borne in mind that the error inherent in the method may be as large as ±28 per cent of the mean, as calculated on the basis of the data recorded in Table III. Consequently, in only a few instances, such as with preparations AS29, C70, C80, and A66 having definitely low or high values, is it possible to assert with any degree of certainty that these products are inferior or superior to the large majority we have tested. The same considerations apply to the results for streptococcal antitoxin. Here, the error of the method is probably about 25 to 30 per cent. In respect to the titers of influenza A mouse protective or neutralizing antibody, great caution must be exercised in drawing any conclusion as to significant differences based only on a comparison of titers between the various preparations, since it is clear from Table III that the error of the method may be as great as 150 per cent in tests carried out at different times. Again similar considerations apply to the results of complement fixation tests mainly because of the difficulty in removing the anticomplementary properties of the fractions. Indeed, under these headings we have included only a few results as the majority of determinations have given endpoints which we regard as untrustworthy from the standpoint of quantitative interpretation. The accuracy of the titrations of H and O agglutinins may be affected by the use of new batches of antigen and by other conditions im-

possible to control; accordingly, the end-point may vary within at least one dilution-interval.

In connection with the assays summarized in Table V, it should be pointed out that, although there was a decrease in α- and β-globulins in preparations made by methods 3A, 3C, 3D, and 3E, which yielded products that contained more than 95 per cent γ-globulin, as contrasted with methods 2 and 3, there is no indication of diminished antibody activity.

TABLE V

Immunologic assay of various preparations of Fraction II

Preparation Number	Titer of solution									Units antitoxin per ml. of solution	
	Isoagglutinin[a]		Anti-comp. act.	Typhoid Agglutinin		Mumps comp. fix.	Influenza A				
	Anti-A	Anti-B		O	H		Comp. fix.	Hirst test	Mouse prot.	Diphtheria[b]	Streptococcus[c]
Method No. 2											
A54R	4	16	640	0	128	(80[d])	(160[d])	512	96	1.9	40
A54K	32	8	32	4	90	(40[d])	(20[d])	256	100	1.5	35
A58	8	8	0	4	128	226	450	512	160	1.7	
Method No. 3											
A66 (stan.)	4	16	>128[e]	9.3[e]	187[e]	80[e]	280[e]	302[e]	144[e]	3.7[e]	50
A72	8	16	20	8	180	[g]	[g]	128	107	1.8	30
A29			16	4	64	[g]	[g]	256	90	1.2	
A35	16	8	>256	2	180	[g]	[g]	256	113	2.7	40
A80R			>256	6	64	[g]	[g]	256	169[h]	1.5	35
A84			>256	6	45	(160[d])	(160[d])	128	116	1.7	25
A74B		4	>256	24	>256	[g]	[g]	256	96	2.8	35
A109			128	16	64	80	320	256	80	4.6	35
C36	16	16		6	128	[g]	[g]	256	106	3.0	
C51	32	8	8	6	45	20	80	256	141[f]	2.4	50
C70	32	8	8	6	45	20	[g]	128	110	1.3	
C80	32	4	>128	4	64	[g]	[g]	256	120	4.0	
C97			>256	12	45	[g]	[g]	128	116	1.5	20
C102			>256	8	45	(320[d])	[g]	256	106	1.5	20
C103			>256	12	90	[g]	[g]	256	137	2.2	30
C104			128	8	45	[g]	[g]	512	137	2.4	20
C105			128	8	45	[g]	[g]	512	160	2.5	35
C106			128	8	45	[g]	[g]	256	60	2.4	30
C107			128	8	64	[g]	[g]	256	120	2.0	65
C108			>256	8	64	[g]	[g]	256	180	2.0	65
C109			16	6	64	[g]	[g]	256	175	2.3	35
D26	64	8	128	4	90	(40)	(40)	256	80	2.8	50
D36	64	16	64	6	90	[g]	[g]	256	98	2.8	40
Method No. 3A											
A74A	8	8	256	12	256	[g]	[g]	256	106	3.8	35
AS84			64	8	90	80	320	256	250	2.4	80
A97			128	8	45	80	640	256	160	1.6	30
B1			128	12	32	40	160	256	215	1.6	
B2			64	6	32	320	320	256	96	2.3	
B3			8	12	32	160	113	128	96	1.8	45
D5			4	22	128	113	450	256	120	3.0	55
D6			16	12	90	113	450	512	192	3.3	20
D8			16	12	64	56	450	512	266	7.5	
D10			128	12	64	56	450	512	267	2.8	
D16			256	12	90	80	450	512	240	3.2	
D20			256	12	64	56	320	512	240	3.1	
G13			>1024	6	32	[g]	[g]	256	267	3.3	
G14			>1024	6	23	[g]	[g]	256	213	2.8	
G15			256	6	16	113	450	512	266	3.3	

TABLE V—*Continued*

Preparation Number	Titer of solution									Units antitoxin per ml. of solution	
	Isoagglutinin[a]		Anti-comp. act.	Typhoid Agglutinin		Mumps comp. fix.	Influenza A				
	Anti-A	Anti-B		O	H		Comp. fix.	Hirst test	Mouse prot.	Diphtheria[b]	Streptococcus[c]
Method No. 3B											
A111			>256	12	64	80	160	128	240	2.8	45
A269K			256	8	64	80	640	128	181	2.8	35
A291K			>256	12	32	160	320	256	45	2.1	20
E1			32	8	64	80	113	256	96	2.2	40
E3			8	6	32	160	160	256	96	1.8	20
E4			512	23	64	113	450	256	267	2.6	
F1			128	12	64	[g]	[g]	256	120	3.7	
F2			16	6	90	56	80	256	69	3.0	
Method No. 3C											
A1–120			256	12	90	[g]	[g]	128		2.8	35
A2–120			256	22	64	[g]	[g]	256	48	1.8	
A1–122			128	12	16	[g]	[g]	256	103	1.8	30
A2–122			>256	12	32	[g]	[g]	32	89	2.0	30
A1–126			>1024	6	32	40	450	512	238	2.3	50
A1–371			256	6	45	40	450	512	200	2.2	35
A2–371			>1024	6	45	113	450	512	236	2.3	35
A1–388			>1024	6	16	40	450	512	160	4.1	40
A1–388B			256	8	32	40	320	512	320	3.5	80
A1–LY1			512	8	22	80	160	512	160	3.7	80
Method No. 3D											
A1–329			128	8	32	113	225	256	120	2.8	60
Method No. 3E											
A1–133			512	12	90	113	450	512	267	2.8	80
Averages	26	10		9	71	98	260	308	150	2.6	41

a. Carried out by Capt. J. Elliott and Lt. L. Pillemer. The titer represents the highest dilution which is found to give a 1+ reaction by test-tube centrifuge technic, using a 1.25 per cent fresh cell suspension.
b. Some of these titrations were carried out under the direction of Dr. G. Edsall at the Massachusetts Antitoxin and Vaccine Laboratory.
c. Carried out under the direction of Drs. A. Wadsworth and M. Kirkbride and Miss J. Hendry at the State of New York Department of Health.
d. After trypsin digestion to remove anticomplementary activity.
e. Average of values given in Table III.
f. Average of two values: 123 and 160.
g. Fixes normal parotid gland or normal egg, and test could not be carried out.
h. Average of 2 values: 125 and 213.

Figures represent reciprocals of end-point dilution of fraction except those for diphtheria and streptococcal antitoxins.

Further evidence is included in Table VI for the close association with γ-globulin of all the antibodies investigated except the O typhoid agglutinin and isoagglutinins. In this table are given the quotients obtained by dividing the value of the end-point of each preparation mentioned in Table V by that of the standard, A66. With the exception of the figures for streptococcal

TABLE VI

Immunologic assay of various preparations of Fraction II

Antibody Concentration Referred to Prep. A66

Preparation Number	Concentration Ratio*										
	Isoagglutinin		Anti-comp. act.	Typhoid Agglutinin		Mumps comp. fix.	Influenza A			Diphtheria Antitoxin	Streptococcus Antitoxin
	Anti-A	Anti-B		O	H		Comp. fix.	Hirst test	Mouse prot.		
Method No. 2											
A48										0.5	1.0
A54R	1.0	1.0								0.5	0.8
A54K									0.6	0.4	0.7
A58	2.0	0.5								0.4	
Method No. 3											
A66 (stan.)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
A72	2.0	1.0		0.7	0.7			0.5	1.1	0.5	0.6
A29			0.5	1.0	1.0	1.0	0.5	1.0	0.6	0.4	
A35				1.0	0.7			1.0	0.8	0.7	0.8
A80R				0.8					1.3	0.4	0.7
A84			0.2	0.5	0.2			0.5	1.2	0.4	0.5
A74B			1.0	2.0	1.0	[a]	[a]	1.0	0.8	0.7	0.7
A109			2.0	1.0	1.0	1.0	2.0	1.0	0.5	1.2	0.7
C36									0.7	0.8	
C51				1.0	0.2	1.0	1.0		1.1	0.6	1.0
C70				1.0	0.2	1.0			1.1	0.3	
C80				0.7	0.4			1.0	1.3	1.3	
C97			>2.0	1.0	0.2			0.5	1.2	0.4	0.4
C102			>2.0	0.7	0.2	0.5		1.0	1.1	0.4	0.4
C103			>2.0	1.0	0.4			1.0	1.4	0.6	0.6
C104			1.0	1.0	0.3	[a]		2.0	0.9	0.7	0.4
C105			1.0	1.0	0.3	[a]	[a]	2.0	1.0	0.7	0.7
C106			1.0	1.0	0.3	[a]	[a]	1.0	0.4	0.7	0.6
C107			0.5	0.7	0.3	[a]	[a]	1.0	0.9	0.6	1.3
C108			1.0	1.0	0.3	[a]	[a]	1.0	1.4	0.6	1.3
C109			0.1	0.5	0.3	[a]	[a]	1.0	1.3	0.6	0.7
D26								1.0	0.8	0.8	1.0
D36				1.0	0.5	[a]	[a]	1.0	1.0	0.7	0.8
Method No. 3A											
A74A			>1.0	1.5	1.0	[a]		1.0	0.9	1.0	0.7
AS84			8.	0.7	0.4	1.0	1.0	2.0	1.3	0.6	1.6
A97			1.0	1.0	0.2	1.0	2.0	1.0	1.1	0.4	0.6
B1			1.0	0.8	0.5	0.5	1.0	1.0	2.7	0.4	
B2			2.0	0.4	0.3	2.7	2.0	1.0	1.0	0.5	
B3			0.5	1.0	0.3	1.0	0.7	0.5	1.0	0.4	0.9
D5			0.3	1.8	1.0	0.7	2.8	1.0	1.2	0.7	1.1
D6			0.5	1.0	1.0	0.7	4.0	2.0	2.0	0.7	0.4
D8			0.1	1.0	0.7	1.0	2.8	2.0	2.0	1.7	
D10			1.0	1.0	0.7	0.7	3.9	2.0	1.4	0.6	
D16			1.0	1.0	0.7	0.7	2.8	2.0	1.3	0.7	
D20			1.0	0.8	0.7	1.0	2.8	2.0	1.5	0.8	
G13			16.	0.5	0.3			2.0	1.5	0.8	
G14			>16.	0.5	0.2			1.0	2.2	0.7	
G15			4.	0.5	0.2	2.0	2.8	2.0	2.0	0.7	

TABLE VI—*Continued*

Preparation Number	Concentration Ratio*										
	Isoagglutinin		Anti-comp. act.	Typhoid Agglutinin		Mumps comp. fix.	Influenza A			Diphtheria Antitoxin	Streptococcus Antitoxin
	Anti-A	Anti-B		O	H		Comp. fix.	Hirst test	Mouse prot.		
Method No. 3B											
A111			>2.0	0.8	0.5	0.5	0.5	1.0	1.5	0.6	0.9
A269K			2.0	1.5	0.3	1.4	[a]	1.0	0.9	0.6	0.7
A291K			8.	1.0	0.3	1.0	2.0	2.0	0.7	0.5	0.4
E1			1.0	1.0	0.5	0.7	0.7	1.0	1.0	0.5	0.8
E3			0.3	0.5	0.5	1.4	1.0	1.0	1.0	0.4	0.4
E4			2.0	1.0	2.0	0.7	4.0	1.0	1.7	0.6	
F1			8.	1.0	0.5			1.0	1.2	0.8	
F2			1.0	0.5	0.7	0.3	0.5	1.0	0.7	0.7	
Method No. 3C											
A1–120			16.	0.7	0.7			0.5		0.6	0.7
A2–120			>8.	1.4	0.5			1.0	0.4	0.4	
A1–122			4.	0.7	0.1			1.0	1.1	0.4	0.6
A2–122			>8.	0.7	0.3			0.1	0.9	0.5	0.6
A1–126			>16.	0.5	0.5			2.0	1.2	0.6	1.0
A1–371			0.5	0.8	0.5	1.4	2.8	2.0	1.4	0.6	0.7
A2–371			>4.	0.5	0.7			2.0	1.3	0.6	0.7
A1–388			>8.	0.5	0.2			2.0	0.8	0.7	1.6
A1–388B			2.0	0.7	0.5	0.7	2.8	2.0	1.5	0.8	2.0
A1–LY1			>8.	0.7	0.2	0.7	1.0	2.0	0.8	0.9	2.0
Method No. 3D											
A1–329			1.0	0.7	0.3	0.7	1.3	1.0	0.6	0.7	1.5
Method No. 3E											
A1–133			4.	1.0	1.4	0.7	2.8	2.0	1.2	0.7	2.0
Averages	(1.5)	(0.9)		0.9	0.5	1.0	1.9	1.3	1.2	0.7	0.9

$$* \text{ Ratio} = \frac{\text{end-point of fraction}}{\text{end-point of standard (A66)}}.$$

a. Fixes normal parotid gland or normal egg, and test could not be carried out.

antitoxin and the isoagglutinins, the standard was titrated simultaneously with each of the preparations.

The figures serve again to emphasize the general uniformity of the majority of the preparations in respect to antibody content. It is neccessary, however, to consider the significance of certain outstanding differences. It might be inferred that the pools of plasmas from which the preparations exhibiting high ratios were prepared contained an originally higher concentration of a particular antibody. In most instances, a correlation of this sort has been noted between the level of plasma antitoxin and that of the fraction. Thus, preparation A109, was found to contain about 4.6 units of diphtheria antitoxin. Its plasma was assayed at 0.25 units. In contrast, preparations A80 and A84 (Tables V, VI, and VII) were assayed at only 1.8 and 1.7 units, respectively, and the corresponding plasma values were determined as about 0.08 and about 0.09 units. The plasma value for diphtheria antitoxin of the

TABLE VII

Immunologic assay of various preparations of Fraction II

Antibody Concentration Referred to Plasma

Preparation number	Concentration referred to plasma									
	Isoagglutinin		Typhoid agglutinin		Mumps comp. fix.	Influenza A			Diphtheria antitoxin	Streptococcus antitoxin
	Anti-A	Anti-B	O	H		Comp. fix.	Hirst test	Mouse prot.		
Method No. 2										
A48	4	4								20
A54R	4	4	1	32			16	16	20	27
A58	1	1	2	32			16	25	39	
Method No. 3										
A66	2	4	2	17	20	18	10	29	34	20
A72	3	4	2	30			16	68	41	20
A80R			<2	16			4	27	23	25
A84			2	<10			4	24	18	
A109			4	16			8	18	18	
Method No. 3A										
A97			2	12			8	31	25	
Method No. 3B										
A111			>3	>16			4	25	20	
Method No. 3C										
A1–120			2	22			4	18	20	
A2–120			3	16			8	5	21	
A1–122			1	8			8	10	30	
A2–122			1	16			1	9	30	
A1–126			2	11			16	21	24	
Method No. 3D										
A1–113								17	27	
A2–113								50	24	
Method No. 3E										
A1–133			2	14			16	10	20	
A2–133			5	11			16	9	17	
Averages	(3)	(4)	2	19	(20)	(18)	10	23	25	22

pool from which the standard (A66) was prepared was not unusually high, and it is therefore impossible on this basis to account for the large amount of antitoxin found in the standard. Possibly the plasma antitoxin was originally higher than indicated by the titration values, since it was allowed to stand at ice-box temperature for some time before it was preserved in the

frozen and dried state and titrations were carried out. One can also think that the processing of A66 differed in some unknown respect from that of other preparations. This possibility, however, seems unlikely.

On the basis of our results and observations of many tests in mice, we are inclined to regard a ratio of 2 in measurements of influenza A neutralizing antibody as signifying that the preparation contains definitely more antibody than the standard. Similarly, ratios of 0.5 or less suggest that the fraction is not equivalent to the standard. We have no definite indication that the plasmas employed in the preparation of certain of the fractions and which have been tested for influenza neutralizing antibody differed significantly in the amount of this antibody and therefore are unable, as was the case in certain instances with the diphtheria antitoxin, to attribute large differences between ratios to this cause. It is possible, however, that repeated testing of plasmas, as well as analysis of a larger number, collected from different geographical areas than we have examined, might in some instances reveal significant differences in the antibody content of successive pools. Certainly, one would expect that pooled blood, collected after the cessation of an epidemic of influenza A, such as now exists in the United States, should exhibit a marked increase in antibody compared with the pools mentioned in this report which were studied before the onset of the epidemic. Indeed, this expectation has been confirmed by the results of titrations on the most recently prepared fractions which are derived from plasma collected subsequent to this epidemic of influenza (see Tables V and VI).

In Table VII, the results of a few direct comparisons of antibody content of plasmas and their corresponding fractions are presented. It is evident that the factors of concentration for the antitoxins, influenzal neutralizing antibody and typhoid H agglutinin, in most instances, are between 20 and 30. Such concentrations are comparable to those obtained for the standard, A66. Data for mumps and influenza complement fixing antibody are not included (except in the case of the standard) as they were regarded as not quantitatively accurate in these instances. The results from titrations of the hemoagglutinin inhibitor give what are probably erroneously low values because of the presence of the non-specific factor which has already been mentioned.

The stability of antibody in Fraction II

From the practical standpoint it was important to ascertain whether antibody associated with Fraction II remained unchanged in amount during (*1*) prolonged storage in the dried state and (*2*) after preservation at elevated temperatures. In Table VIII are included the results of titrations on 3 preparations, 2 of which had been kept at room temperature during the course of 9 months, the third having been preserved in the ice-box at 0° C. The titrational data are also presented which were recorded on the basis of tests carried out before storage was inaugurated and shortly after the processing was completed. It is apparent that storage under either condition did not significantly reduce the titer of any of the antibodies mentioned in the table. The results for complement fixing antibodies are not given, since again they were regarded as quantitatively unreliable, but they do not suggest that loss of activity has occurred. The figures recorded in Table IX, which were obtained from tests on preparations which were heated for 2, 4, and 14 days, respectively, at 50° C., indicate that little or no loss of antibody activity took place under these conditions.

TABLE VIII

Immunologic assay of Fraction II after storage as dried powder

Preparation number	Time (months) and temp. of storage	Titer of solution: Typhoid agglutinin O	Typhoid agglutinin H	Influenza A: Hirst test	Influenza A: Mouse prot.	Units diphtheria antitoxin per ml. solution
A29	0	4	64	256	90	1.2 a
A29	9 (RT)	8	128	256	64	2.0
D26	0	4	90	256	80	2.8
D26	9 (RT)	<4	64	256	106	2.3
D36	0	6	90	256	98	2.8
D36	9 (0°)	6	64	512	193	2.7

a. Early titration value uncertain.

Although the data are few, it is clear from these experiments on the effect of storage and

TABLE IX

Immunologic assay of immune serum globulin solutions after heating at 50° C.

Preparation number	Time and temp. of storage	Titer of solution				Units diphtheria antitoxin per ml. solution
		Typhoid agglutinin		Influenza A		
		O	H	Hirst test	Mouse prot.	
A54R	0	0	128	512		1.9
A54R	44 hours	4	128	256		1.2 a
A35	0	12	180	256	113 b	2.7
A35	96 hours	4	180	256	80	2.7
A35	14 days	6	64	512		2.6

a. Value may be too low because of titration interval.
b. Average 3 values—67, 60, and 213.

heating that the antibodies of Fraction II, for which tests have been made, are stable under conditions to which they would be subjected if employed in the civil or military practice of medicine.

The antibody content of Fraction III-1

It will be recalled that Fraction II was developed by subfractionation of Fraction II + III. A 12 per cent solution of the latter will occupy only one-tenth the volume of the plasma from which it was prepared, and hence would show a maximum concentration of about 10 times. Fraction III, obtained by the separation of Fraction II, has been separated into 2 subfractions designated III-1 and III-2. On the basis of its volume compared to the plasma from which it was obtained, a 20 per cent solution of Fraction II would show a maximum antibody concentration of about 40 times that of the plasma and Fraction III-1 about 25 times. Fraction III-2 containing the prothrombin and complement component *c'*1 (1), consists of a small fraction of the proteins composing Fraction II + III.

Although the 15- to 30-fold concentrations of antibody over plasma obtained by the isolation of Fraction II are remarkably high, as judged by a comparison of the results obtained by a variety of methods which have previously been employed in the concentration of immune sera, it was apparent from the factors of protein concentration just mentioned that considerable quantities of antibody were either inactivated during the process or were recovered in Fractions III-1 or III-2. That no significant amount of antibody was carried over into Fraction IV will be recalled from the findings previously set forth. It became of interest, therefore, to assay immunologically a few specimens of Fraction III-1. The results obtained with 2 preparations and recorded in Table X indicate that much of the

TABLE X

Immunologic assay of 2 preparations of Fraction III-1

Preparation number	Antibody concentration referred to plasma				
	Typhoid agglutinin		Influenza A		Diphtheria antitoxin
	H	O	Hirst test	Mouse prot.	
III-1 A80	12	21	5	12	5
II A80	16	1	4	27	20
III-1 A84	> 6	6	5	15	0.7
II A84	>16	2	4	24	30

antibody not accounted for in Fraction II is concentrated in Fraction III-1. Thus, on the basis of the protein concentration factors mentioned above, it can be calculated that considerable amounts of the influenza mouse protective antibody were found in Fraction III-1 of A80 and A84.[10] The distribution of influenza hemoagglutinin is similar to that of the mouse protective antibody. Again in this case, the relatively low values are to be attributed to the presence of the non-specific factor, we believe. About 20 per cent or less of the available diphtheria antitoxin may go into the III-1 moiety and somewhat over half the H typhoid agglutinin. As was expected, because of the low value of Fraction II for O agglutinin, nearly all of this antibody was shown to be associated with III-1. The fact that a marked separation of O agglutinin was secured, in contrast to the behavior of the other antibodies, affords further evidence that it is probably not associated with the same species of protein as the latter.

[10] The yields of gamma globulin should decrease in Fraction III-1, and increase in Fraction II, as improvements are made in the theory and practice of this separation.

DISCUSSION

The plasma fractionation method being employed in the processing of Red Cross blood has yielded Fraction II, a concentrate of the normal human γ-globulins in which a fairly representative group of antibodies has been separated from the other proteins of human plasma.

In the case of neutralizing antibody for influenza A virus, the potency of Fraction II appears to approximate that of the average of sera taken during early convalescence from the disease. It will be recalled that the mean titer of the 29 preparations of Fraction II mentioned in Table V was 1:129. Seven preparations, however, have exhibited titers of 1:160 or over, and 11, of 1:130 or over. Horsfall (8), using essentially the same technic as ourselves, obtained a mean titer of 1:209 for convalescent sera, drawn on the average 30 days after the onset of the disease. This figure is perhaps somewhat too high to be representative of the true mean of convalescent sera studied by him since it is derived from the data obtained in 4 epidemics, in 3 of which the mean titers were 1:61, 1:130 and 1:194 respectively, whereas, in the fourth, the mean titer was 1:424.

When the titers of complement fixing antibody in sera taken from individuals recently convalescent from influenza A are compared with the end-points obtained with Fraction II, it is found that the latter again approach the average convalescent serum in concentration of this antibody. For example, in a recent epidemic of influenza A in Boston, the mean titer of sera drawn from 20 individuals on the average of 18 days following the onset of symptoms was 1:363.[11] This figure does not widely differ from the average titer of complement fixing (1:280) antibody, determined in the case of the standard globulin preparation (Table III).

In contrast to influenza A, the concentration in Fraction II of the complement fixing antibody of mumps is definitely less than that occurring in most convalescent sera. The mean final titer[12] of mumps complement fixing antibody in 33 sera drawn during the third and fourth weeks of convalescence has been determined as 1 : 1300.[13] The average final titer of this antibody in the standard (Table III) was 1 : 240 (*i.e.*, 3 times the initial titer given in the table). The most probable explanation for this difference between the amounts of influenzal and mumps complement fixing antibody in the fraction may lie in the fact that the quantity of mumps antibody in plasma is on the average less than that of influenzal antibody. As indicated by the results from comparative determinations on 6 pools of plasma, the mean titer of the influenzal antibody is about 4 times greater than that of the mumps antibody.

Very little information is available on the antitoxin content of the serum in those convalescent from untreated diphtheria, since the administration of antitoxin has been generally employed for many years. The largest number of such determinations which we have been able to find recorded are those of Schürer (9) who examined the sera of 18 convalescents, drawn between the 25th and 60th day after onset. In this group, the average was 0.13 unit per ml. Individual sera varied from 0.02 unit to somewhat greater than 1 unit in content of antitoxin. It is evident, then, that the preparations of Fraction II (Table V) contain on the average about 10 times the quantity of antitoxin which is usually present in convalescent sera. But in this connection it should be pointed out that the low antitoxin content of the diphtheria sera is exceptional. In most other acute infectious diseases, the serum of the recent convalescent generally contains many times the quantity of antibody which is characteristic of the normal serum (10).

As with the data for levels of diphtheria antitoxin in the sera of recent convalescents, so the

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[11] The titrations on which this figure is based were done partly in the laboratory of the Department of Bacteriology and Immunology at the Harvard Medical School and partly in the laboratory of the *Thorndike Memorial*, Boston City Hospital, by Dr. Maxwell Finland and his associates to whom we are indebted for permission to use their data.

[12] We have employed final dilutions in computing the average for convalescent sera because the volumes employed in our earliest tests on convalescent sera were not the same as those subsequently used.

[13] This relatively high value is the average of unpublished data obtained in the Department of Bacteriology and Immunology, Harvard Medical School. On the other hand, two pools of mumps convalescent sera collected for fractionation at the Harvard Plasma Fractionation Laboratory from about 400 donors have final titers of 1 : 240 and 1 : 480.

recorded information concerning the amount of streptococcal antitoxin in those just recovered from scarlet fever is scanty. Rhoads and Gasul (11), in 12 pools of convalescent sera, found the average titer of the pools to be about 10 units per ml. These authors do not mention the number of individual sera of which each pool was composed. For purposes of comparison, they also determined the average titer of 3 pools of normal adult sera and found it to be somewhat less than 5 units per ml. Moore and Thalhimer (12), in a study of 51 convalescent sera, found the average titer to be 3.3 units per ml. This figure is similar to the results obtained earlier by Henry and Lewis (13). Thus, a comparison of the titrational values of Fraction II for streptococcal antitoxin (Table V) with these results which have been recorded in the literature shows that the globulin concentrates contain on the average more than 3 times the quantity of antitoxin. Even the preparations exhibiting the lowest titers are at least twice as potent as the average convalescent serum.

The concentration of the H typhoid antibacterial antibody in Fraction II is, on the average, definitely less than that ordinarily encountered during the latter stages of typhoid fever and convalescence therefrom. Thus, Gardner and Stubington (14), in a series of 40 typhoid patients, obtained titers of over 1:1000 in 14, and over 1:128 in 23. Only 3 cases exhibited titers of less than 1:128. The average was 1:2130. The mean of our preparations of Fraction II was 94 (Table V). In 6 instances, however, titers were recorded comparable to those found for certain of the sera studied by Gardner and Stubington.

The O antibody level in Fraction II, as we have pointed out, scarcely exceeds that of the pooled plasma, since this antibody is separated from the others and recovered in Fraction III-1. There the amount of O antibody in the few specimens which have been titrated is considerably less than that found by Gardner and Stubington in their 40 sera, the average titer of which was 1 : 906.

A few determinations of the titer of Fraction II in protective antibody against infection of mice with *E. typhosa* have been carried out by Major G. S. Luippold at the Army Medical School. The results revealed antibody levels comparable to those characteristic of individuals who recently have received the standard course of typhoid vaccines.

Since, in a number of infectious diseases, convalescent serum has been successfully employed as a therapeutic or prophylactic agent, we have carried out the foregoing comparisons with the purpose of securing some indication as to whether Fraction II on *a priori* grounds might prove as effective in such applications as the serum. It is clear that no general conclusion in this respect can be drawn. In certain instances, the fraction may exceed or equal the potency of convalescent sera; in others, it falls definitely below the average level of convalescent antibody. In no case, however, where the data are adequate, save in that of the O typhoid antibody, is the factor of difference in potency of the average preparation of Fraction II and the convalescent serum much over 4 or 5.

These comparisons also serve to emphasize the expediency of titrating each newly prepared lot of Fraction II along with a standard preparation, in order to ascertain whether or not it bears the same general relation in respect to a variety of antibodies to convalescent sera as the standard. Since it has not yet been demonstrated that Fraction II is of value in any disease save measles, and since we are unable to measure the concentration of measles protective substance directly, the reader may be at a loss to appreciate the necessity for this recommendation which involves an assay of entirely unrelated antibodies. It would seem, however, to be the only manner by which we can, in our present state of knowledge, obtain at least presumptive evidence that the measles antibody (or any other antibody for which laboratory tests cannot be made) is present in adequate amount. We make this statement because of the following considerations. The standard has been shown to be effective by clinical test in the prevention and modification of measles (15, 16). The levels of the various antibodies in pooled adult plasma obtained in the northeastern, midwestern, and Pacific sections of the United States appear on the basis of our findings to show relatively little variation. Consequently, it seems logical to think that the quantity of antibody for measles virus should

not vary greatly in the plasma pools and should be concentrated in Fraction II to the same degree as those reacting with the antigens for which we have tested, in particular the viruses of influenza A and mumps.

If this hypothesis be correct, it is clear that a given preparation of Fraction II which is shown to be markedly inferior in potency to the standard should not be employed. It is, of course, possible that as experience is gained from trial in the field that the procedure adopted in the present investigation for estimating the value of preparations of Fraction II can be modified in the direction of simplification by eliminating tests for certain antibodies.

In respect to the theoretical problems raised at the beginning of this communication, it must be frankly stated that we cannot as yet give definitive answers. Thus, the evidence presented does not warrant the statement that the antibodies of human plasma are exclusively associated with the γ-globulin. Our findings, however, strongly suggest that the antibodies reacting with 2 viruses (or their products), diphtheria and streptococcal antitoxin, and the H antibody for *E. typhosa* are γ-globulins. This is indicated by the fact that 50 per cent or more of these antibodies can be recovered in certain preparations of Fraction II which contain 95 to 100 per cent γ-globulin. Furthermore, no decrease was observed in the concentration of these antibodies in Fraction II as the percentage of γ-globulin was increased by appropriate procedures from 80 to 100 per cent. The observed antibody concentrations over plasma are also very near that calculated for the concentration of γ-globulin in these solutions.[14]

The nature of the protein associated with the O typhoid antibody and isoagglutinins is less certain since these antibodies could be almost quantitatively recovered in Fraction III-1 which contains approximately 3 per cent α- and 70 per cent β-globulin in addition to 25 per cent γ-globulin. Because of the large amount of γ-globulin present in III-1, it is entirely possible to believe that these antibodies are included therein. But if this be so, it would seem that the γ-globulin with which they are bound is probably different from that associated with antibody in Fraction II because of the separation of these antibodies from the others. There is no evidence, however, to eliminate the possibility that the O antibody and the isoagglutinins are included in either the α- or β-globulins or indeed in both of them.

Because of the nature of the antigens involved, the difference in chemical behavior of these two antibodies naturally gives rise to speculation. Our knowledge of the nature of the antigens homologous for the various antibodies with which we have dealt indicates or suggests that both the toxins, the two viruses, and the H typhoid antigen are composed largely or entirely of protein and that the O typhoid and the isoagglutinins are complex substances consisting, in part, of polysaccharides. Accordingly, it is perhaps not illogical to consider the possibility that antigens of the latter type react with antibodies which in turn can be distinguished by differences in their physicochemical properties from those which are specific for simple protein antigens. Before any correlation of this sort can be definitely asserted, it is clear that many more antigenic species and their corresponding antibodies must be investigated.

In concluding this discussion, we would again emphasize the following facts of practical value which the data have revealed. A concentration of about 15 to 30 times of certain of the normal antibodies of human plasma has been obtained by the separation and purification of the γ-globulins. A large number of preparations of Fraction II, produced both at Harvard and by manufacturers elsewhere, from pools of plasma obtained in several areas throughout the United States, have been tested for their content of 6 different antibodies and a satisfactory degree of uniformity between these various preparations has been demonstrated.

CONCLUSIONS

1. The fractionation of normal human plasma collected by the American Red Cross yields a

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[14] Plasma contains about 6.6 grams of γ-globulin per liter. If we standardize on a γ-globulin concentration of 165 grams per liter in our final Fraction II solution, this yields a concentration factor of 25 times. Such a solution will contain 16.5% protein if Fraction II is 100% γ-globulin, but must be more concentrated if the purity is lower, a 20% solution of Fraction II containing 82% γ-globulin yielding about the same concentration factor.

gross fraction comprised of α-, β- and γ-globulins designated Fraction II + III which contains a large proportion of the antibodies reacting with a variety of pathogenic bacteria and their products, viruses, and the iso-antigens of the human blood groups concentrated from 4 to 10 times.

2. The normal human γ-globulins have been further separated and concentrated in Fraction II. This has been found on immunologic assay to contain antibodies reacting with diphtheria toxin, streptococcal erythrogenic toxin, influenza A virus, mumps virus, and the H antigen of *E. typhosa*. These antibodies were concentrated from 15 to 30 times as compared to pooled plasma.

3. In solutions concentrated to at least 25 times the plasma pool Fraction II gives titers of certain antibodies comparable with or greater than those of the corresponding convalescent sera. In other cases, the potency of Fraction II is somewhat lower than the convalescent serum, but not by a factor of more than 4 or 5.

4. Antibody reacting with the O typhoid antigen was present only in low titer in Fraction II but was recovered in large amounts in Fraction III-1,—another derivative of Fraction II + III.

5. Immunologic assay of the antibodies in 62 preparations of Fraction II, derived from plasma collected in various sections of the United States, has revealed a general uniformity of potency. There has, however, been an increase in influenza A antibodies following a recent epidemic of the disease.

6. The titer of the antibodies of Fraction II after prolonged storage and exposure to moderately elevated temperatures is not significantly reduced under the experimental conditions described.

7. The implications of these findings to the standardization of Fraction II as an agent in the prophylaxis and therapy of disease are discussed, together with their significance in respect to the nature of normal human antibodies.

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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

XI. THE USE OF CONCENTRATED NORMAL HUMAN SERUM GAMMA GLOBULIN (HUMAN IMMUNE SERUM GLOBULIN) IN THE PROPHYLAXIS AND TREATMENT OF MEASLES[1,2]

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INTRODUCTION

Human immune bodies, obtained from the blood of individuals who have had measles, when injected parenterally in exposed susceptibles in sufficient quantities and at a suitable interval from the time of exposure, have long been recognized as useful in attenuating or in passively protecting against measles. In the process of fractionation of pooled human plasma, developed for the production of normal human serum albumin by Cohn, Oncley, Strong, Hughes and Armstrong (1), fractions became available in which a variety of antibodies, reacting with certain viruses and bacteria, were found by Enders to be concentrated many times (2). It became obvious that such concentrated preparations of antibodies should be useful against those diseases for which the attenuating, protective, or curative value of human immune plasma or sera had been demonstrated. The present report presents: A. A study of the use of such globulin fractions of human plasma for attenuation or passive protection against measles in 891 individuals who, as far as could be determined, were exposed susceptibles. B. A study of the use of globulin fractions containing the largest proportion of immune bodies (chiefly gamma globulin) in the treatment of 61 individuals during the early stages of measles.

A. PASSIVE IMMUNIZATION

Materials used for prophylaxis

Two globulin fractions were used in these studies, Fraction II + III in the early work and Fraction II for most of the period of investigation. In Fraction II + III, the first crude concentrate containing all the gamma globulins and considerable beta globulin (1), Enders found the theoretically expected 8 to 10-fold concentration of many antibodies present in normal pooled plasma (2). In Fraction II, the gamma globulins and antibodies were further purified (1), so that the titer of those antibodies which appeared in this fraction (2) was from 15 to 35 times as high as in the original plasma pool.

In view of the concentration of immune bodies reacting with viruses in Fraction II, it appeared that the value of this fraction in measles should receive the more intensive study. Thus, a few preliminary tests were made of the value in measles of Fraction II + III, while the bulk of the data collected concerns the studies of Fraction II.

Clinical material

A measles epidemic, occurring through the winter of 1942 to 1943 and the spring of 1943 in the Philadelphia area, afforded the opportunity for a study of the value of the globulin fractions. Also, in the spring of 1943 in the Baltimore area, a measles epidemic appeared which gained little headway during the summer. However, in both areas, the disease was of considerably greater than average severity, —an opinion based first upon the relatively high incidence of cases of measles encephalitis; second upon the ease and severity with which monkeys contracted the disease when injected with blood from acute cases (3); and third, upon the high fever, extent of the rash, and other clinical signs and symptoms present. Most of the children studied resided in the Philadelphia area, while a smaller group was included from Baltimore. In the latter area, the results were obtained by one of the authors (S. S. G.); while in

[1] These investigations were aided in part through the Commission on Measles and Mumps, Board for the Investigation and Control of Influenza and other Epidemic Diseases in the Army, Preventive Medicine Division, Office of the Surgeon General, United States Army.

[2] The products of plasma fractionation employed in this work were developed from blood, collected by the American Red Cross, by the Department of Physical Chemistry, Harvard Medical School, Boston, Massachusetts, under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

Philadelphia, with the exception of those injected by the authors, the children studied were private patients, either of members of the Medical Staff of the Children's Hospital or, with a few exceptions, of physicians associated with this Hospital.[3]

Inasmuch as attenuation rather than complete protection was usually desired, the continuous central tabulation of the results obtained from the physicians in the Philadelphia area permitted in general a uniform reduction in the amount of globulin they injected during the course of the epidemic. A similar reduction in dosage resulted in the Baltimore area. While, in this manner, the children injected early in the study were most frequently protected from measles, with the reduction in dosage, attenuation was frequently obtained and the amounts which offered the best chance of obtaining either result were thus estimated more accurately.

The injections were intramuscular. The physicians were encouraged to inject susceptibles who were intimately exposed in their homes (not at school or out-of-doors) and within 7 days of the time of exposure. Control groups were obtained in 3 schools, a large number of homes, and 1 institution (Table I). In the 3 schools, 54 children, or 68 per cent of 79 exposed children who gave no history of measles, developed the disease. In the homes, over 90 per cent of the exposed susceptibles with negative histories contracted measles, while in the 1 institution, 43 of 44 children studied developed measles. Because of the severity of the epidemic, it was deemed inadvisable to request families to permit certain of their children to remain unprotected as controls.

TABLE I

Incidence of measles in control children in three private schools—Philadelphia 1943

Total enrollment	170	
Untraceable	2	
Revised total		168
Previous measles	79	
Received passive immunization	7	
Unexposed	3	
Total non-susceptibles		89
Total susceptibles		79
Contracted measles. Winter 1943		54
Failed to contract measles on exposure		25
Contracted measles		68 per cent
Failed to contract measles		32 per cent

[3] The largest groups of children in the Philadelphia area were furnished by Drs. Charles H. Classen, S. Emlen Stokes, Howard S. Curtis, John P. Scott, and Jeannette Munro. In addition, groups of appreciable size were furnished by Drs. Harriet Felton, Sherman Little, Julian M. Lyon, James Reilly, Joseph Ritter, and Elizabeth Rose. Smaller groups were studied by Drs. Charles C. Chapple, Arthur M. Dannenberg, Charles Munro, Arthur Peacock, Stuart Polk, Milton Rapoport, and Mitchell Rubin.

Results

The total number of individuals injected with both globulin fractions, either for attenuation or for complete protection, amounted to 891. A few of this group were adults who professed never to have suffered from measles and who usually requested a sufficient quantity of globulin to insure complete protection. A considerably larger number of children were injected well after the seventh day from exposure with an amount of globulin which could not have been expected either to protect them or to attenuate the disease. Because of the insistence of worried parents, it is frequently impossible for the physician to avoid the use of globulin in children for whom it is not indicated because of early exposure. The number of children injected with Fraction II + III was not sufficient to permit quantitative determinations of the amounts required for protection and attenuation, respectively. Accurate estimates concerning the value of this fraction were particularly difficult, in view of the fact that it was the first fraction tested and usually larger amounts than necessary were injected.

The terms used to describe measles in the injected groups were (*1*) no measles, (*2*) very mild measles, (*3*) mild measles, and (*4*) unmodified measles. The first and fourth categories were clear-cut and, on the whole, easily estimated. Very mild measles was estimated primarily by means of a temperature which did not rise above 101° F., or which had a single spike of temperature on one day, rising above 101° F. but not above 102° F. In such cases, also, the rash was usually sparse, the respiratory signs and symptoms were slight, and the patient was not acutely ill. For the category of mild measles, the temperature remained below 103° F. and other signs and symptoms of measles were obviously mild. The number of cases in this category fortunately were few, since most children could be considered as falling into the second category of very mild measles. An over-all summary of the results which includes all cases injected, without reference to the age, time or type of exposure, or amount of globulin used, is included in Table II. In this summary, the lack of knowledge at first of the excellent protective value of the globulin

TABLE II

Final results in individuals to whom gamma globulin was given as a prophylactic measure against measles—Winter 1943

	Number	Per cent	Number	Per cent	Number	Per cent
No measles	618	69.40	618	71.36	866	97.2
Very mild measles	191	21.44	248	28.64		
Mild measles	57	6.36				
Unmodified measles	25	2.80			25	2.8
Total	891	100.0	866	100.0	891	100.0

is evident in the large number of individuals who suffered no measles, while the insistence of families upon its use, despite too early an exposure, is evident in the group of unmodified cases. Also, the inclusion of casually exposed individuals in this table, although not a large group, conveys a false impression of the number who were completely protected by the globulin.

For the foregoing reasons, it was necessary to select from the entire tabulation those individuals for whom rigid criteria of age, type of exposure, time of exposure, and amount of globulin could be fulfilled. In order to fulfill such criteria, two age groups of children were selected. The first was a pre-school group from 1 to 5 years, inclusive, which was considered as 100 per cent susceptible. The age range of the second group was 6 to 12 years, inclusive. These children also were suceptibles as far as could be determined, although the history of absence of measles at this age-range is somewhat less reliable. In Figure 1 are indicated the age ranges of the children within the two groups selected. Home exposures only were included and only children in whom the globulin had been injected within 7 days of exposure. The percentages of no measles in these two age groups of children are plotted against the amount of globulin injected to form the two curves shown in Figure 2. These two curves are not extended to the right beyond the dosage of 2 and 2.5 cc., nor to the left as far as the dosage of 0.25 cc., because there were not sufficient children in these ranges and beyond to furnish data which were statistically valid. However, the small amount of data available suggested that the curves would ascend rapidly towards the 100 per cent point, inasmuch as a group of 39 out of 40 individuals of all ages, injected with 4 or 5 cc. and fulfilling the criteria of time and type of exposure, did not contract measles. The complete data from which Figure 2 is derived are shown in Table III.

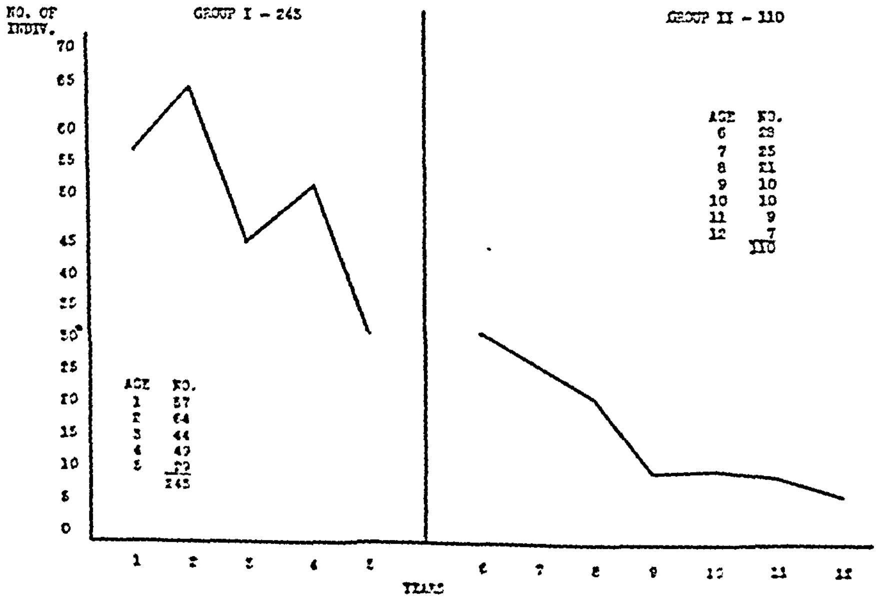

FIG. 1. PROPHYLACTIC ADMINISTRATION OF GAMMA GLOBULIN FRACTION II AGAINST MEASLES—AGE INCIDENCE OF SELECTED GROUPS

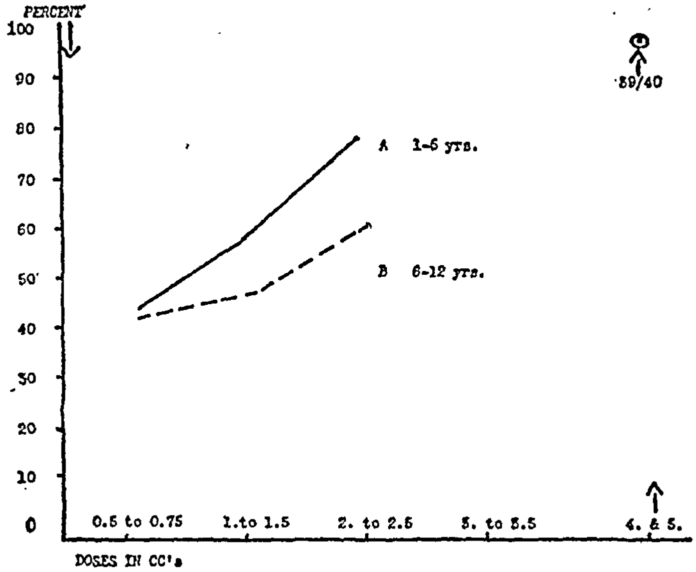

FIG. 2. PERCENTAGE OF NO MEASLES VERSUS MILD MEASLES IN RELATION TO GRADED DOSES OF GAMMA GLOBULIN GIVEN TO SELECTED GROUPS

Discussion

The striking value of Fraction II of the immune globulin for protection against measles is evident from the foregoing data. That it is the most potent product thus far developed for this purpose is also evident, inasmuch as doses of 0.5 cc., when injected within a suitable period in susceptibles exposed at home and in both the age ranges studied, afforded approximately an equal chance of no measles or of attenuation. When the dose was increased to 2 to 2.5 cc. in the same age ranges and under the same conditions, the chances of no measles were approximately from 66 per cent to 80 per cent. Also at this dosage level, with almost no exceptions (2 out of 70), the children who were not completely protected had measles which was considerably attenuated.

The curves of Figure 2 demonstrate clearly a finding which cannot be too strongly emphasized in studies of measles prophylaxis, namely, that within a rather wide range of dosage, protection or attenuation still remains a matter of chance. In such studies, this chance can be estimated with considerable accuracy for a group of children, but the physician cannot as yet give the answer to the parents of the exposed susceptible as to whether or not their child will have attenuation or protection within a specified range of dosage. The reason for such difficulty in the selection of the amount of globulin would appear

TABLE III

Analysis of protection against measles afforded by gamma globulin in selected groups—1943

	0.5 and 0.75 cc.		1.0 and 1.5 cc.		2.0 and 2.5 cc.		3.0 and 3.5 cc.		4.0 and 5.0 cc.		Totals	Per cent
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent		
Ages 1 to 5 years												
No measles	39	45	51	57	39	80	8	67	4	100	141	59.5
Very mild and mild measles	48	55	35	43	9	20	4	33	0	0	96	40.5
Total	87	100	86	100	48	100	12	100	4	100	237	100.0
Ages 6 to 12 years												
No measles	15	44	16	47	12	60	9	60	3	75	55	51.4
Very mild and mild measles	19	56	18	53	8	40	6	40	1	25	52	48.6
Total	34	100	34	100	20	100	15	100	4	100	107	100.0
Over 12 years												
No measles	8	89	4	100	6	75	5	71	32	100	55	91.7
Very mild and mild measles	1	11	0	0	2	25	2	29	0	0	5	8.3
Total	9	100	4	100	8	100	7	100	32	100	60	100.0

Measles total in age groups under 13 years

No measles	196— 57 per cent
Very mild and mild measles	148— 43 per cent
Totals	344—100 per cent

to lie in the difference in susceptibility of individual children. This difference appears to be clearly indicated when a large family of susceptibles, similarly exposed, suffer measles of varying severity. In such a consideration, it is assumed that the virus strain in a single epidemic remains the same.

Despite the inability to determine for each child the size of dose which will protect or attenuate within the dosage range shown in Figure 2, nevertheless when doses are selected either to the left or right of the curves indicated, *i.e.*, below 0.25 cc. or above 4 to 5 cc., the parents can be given considerable assurance as to whether or not measles will occur in their exposed susceptibles. The same criteria obviously also apply to adult susceptibles. In general, therefore, the amounts necessary for protection and for attenuation may be outlined as follows:

	For attenuation	For protection
Children 5 years and under	0.25 to 0.5 cc.	2.0 cc. to 2.5 cc.
Children 6 to 12 years, inclusive	1 cc. to 1.5 cc.	4.0 cc. to 5.0 cc.

Again it must be emphasized that these are approximate figures, due to the difference in susceptibility of individuals.

In any attempt to outline the dosage per pound of body weight, a greater number of cases must be collected. Approximate estimations of proper dosage per pound of body weight, at all ages up to 12 years, are as follows: For attenuation, 0.02 cc. per pound; for protection, 0.08 cc. per pound. It is interesting that these results are in close agreement with those of Ordman, Jennings, and Janeway, as recorded in the following paper in this series (4).

The advantages of the gamma globulin fraction of plasma, used in these studies, above other preparations of human immune bodies lie primarily in the small size of the dose required, the absence of materials other than those present in plasma, the absence of discomfort or reaction at the site of injection, and the absence of any generalized reaction. When this fraction is obtained from large pools of blood, such as those of the American Red Cross, there is in general a uniformity of antibody titer. Differences in the various samples of Fraction II in these studies were not noted, although the nature of the studies prevented the determination of moderate changes in antibody titer. The general impression was gained, corroborated by the laboratory studies of Dr. John Enders and his co-workers, that Fraction II + III required a larger dose for the same effect as that obtained by Fraction II.

In a few samples of Fraction II, "burning" at the site of injection was noted. This infrequent reaction appeared to depend, at least in part, upon a low pH, 6.1 to 6.2, of certain early preparations. The pH has now been standardized at pH 6.8 ± 0.4.

A final answer to the question of dosage depends upon the well-recognized variables which should again be emphasized.

(*1*) Age or weight of individual.
(*2*) Intimacy of exposure.
(*3*) Length of time from first exposure.
(*4*) Susceptibility of the individual, which probably includes the season of the year. In summer, individuals appear to be less susceptible, although no conclusive data are available concerning this question.
(*5*) The desire for complete protection or for attenuation. The general health of the child, the presence of other infectious diseases in the household or the community, the requirements of the social environment, activities, travel, convenience, etc., are all important considerations.
(*6*) The potency of the prophylactic agent used, particularly as related to improper handling by the physician or distributor.

When such variables are given full consideration by the physician, the proper dose of globulin Fraction II for the individual case may be obtained from such observations as are recorded in Figure 2 and Table III. In any final decision, the variable chances of complete protection or attenuation should be explained to the parents or the patient over the range of dosage thus indicated.

Summary

The serum gamma globulin, separated and concentrated by chemical fractionation of normal human blood, has been used in studies on measles prophylaxis. Two fractions, II + III, and Frac-

TABLE IV

Gamma globulin in treatment of measles—1943—a comparison of treated and untreated children

PRE-RASH					RASH				
Initials	Age	Dose	Result*	Contact Controls	Initials	Age	Dose	Result	Contact Controls
	years	*cc.*				*years*	*cc.*		
F. R.	12	15	GM	Not known.	S. E.	2½	10	UM	Very severe.
W. R.	9	15	SM	Not known.	M. L.	7	15	SM	Not known.
J. B.	7	13	SM	Not known.	L. S.	3	7	SM	Mod. severe.
B. P.	7½	15	UM	4 very severe.	K. F.	7	8	GM	Not known.
P. J.	5½	10	GM	Not known.	L. W.	6½	12	GM	Not known.
S. S.	4	12	GM**	Not known.	G. C.	5½	10	UM	Not known.
N. U.	3	5	GM	Not known.	A. L.	17	30	UM	Not known.
J. W.	6	5	UM	Not known.	B. McD.	17	25	SM	Not known.
B. A.	4	10	GM	Moderate.	N. A.	7	10	UM	Not known.
S. DeY.	6½	15	UM	Not known.	R. McC.	1	7½	UM	Mild.
C. T.	8	15	SM	Mild.	P. S.	13	10	UM	Average case.
C. W.	10	10	UM	Mild.	R. D.	10	10	UM	Not known.
G. C.	3½	12	GM	Sibling, average.	A. H.	7	10	UM	Not known.
P. C.	1½	8	GM	Sibling, average.	C. H.	1½	10	GM	1 average.
J. C.	5½	12	GM	Sibling, average.	S. R.	8	15	UM	Not known.
J. A.	7	5	UM	Severe.	A. P.	7½	9	SM	Not known.
W. B.	34	10	SM	Moderate.	G. R.	7	15	UM	Not known.
E. A.	8	10	UM***	Not known.	L. T.	5½	10	UM	Not known.
D. P.	6	5	GM**	Sibling, average.	J. K.	6	5	UM	Not known.
D. P.	6	5	GM	Not known.	Mrs. N.	25	35	UM	Not known.
M. D.	4	10	SM	Not known.	T. L.	3	20	UM	2 average.
P. R.	6½	5	UM	Not known.	D. V.	6	20	SM	2 average.
C. F.	2½	12	SM	Not known.	B. N.	7	20	SM	2 average.
A. B.	2	20	GM	2 average.	B. P.	5	20	SM	3 severe.
A. G.	5	20	GM	1 average.	W. J. W.	6	20	UM	2 average.
N. S.	2	20	GM	1 average.	J. B.	11	20	UM	1 severe.
G. M. A.	10/12	20	SM	3 average.	L. K.	5	20	GM	1 average.
L. D.	4	20	GM	1 average.	P. J.	7	20	UM	2 average.
J. G.	4	20	GM	2 average.	J. F.	5	15	GM	2 average.
T. N. D.	6	20	GM	4 average.	L. T.	4	10	GM	1 severe.
A. L.	3	20	GM	3 average.					

* SM—Slightly modified. UM—Unmodified. *** Severe case.
GM—Greatly modified. ** No rash developed.

tion II, were used. Sufficient data for valid conclusions were obtained with Fraction II. With strict criteria of age, time of exposure, and amount of globulin, curves demonstrating the percentage of no measles of two age groups over a range of dosage were developed. The striking value of Fraction II of human globulin for protection against and attenuation of measles was evident. For size of dosage and ease of injection, gamma globulin Fraction II surpasses other types of human immune bodies.

B. TREATMENT

Introduction

The use of large amounts of convalescent measles serum in the treatment of measles during its early stages has been studied by Levinson (5). The generally favorable results obtained by these workers have been confirmed by a few unpublished records from the Philadelphia Serum Exchange of the Children's Hospital of Philadelphia and from other workers. Clearly demonstrable attenuation of the disease in such studies has occurred only when the convalescent serum has been injected in large amounts intravenously at the time of appearance of Koplik spots and before the appearance of any rash.

The demonstration of a high concentration of immune bodies against measles in the gamma globulin Fraction II of pooled human plasma, as indicated previously in this report, suggested the possible value of large amounts of this fraction injected intramuscularly[4] for the treatment of measles in its early stages. Part B of the present report records the results of such studies.

[4] This fraction of gamma globulin has been released for intramuscular use only. The preparation of an immune serum globulin fraction suitable for intravenous use is being attempted at present.

Material used for treatment

Fraction II + III was used for 8 children early in this study, while Fraction II, in view of its high titer of anti-influenza A antibody and its demonstrated value for protection against or attenuation of measles, as outlined in Part A, was later selected for 53 children. The amount of gamma globulin available was somewhat limited during the major part of the studies on prophylaxis and until a valuable body of data was obtained in the studies of Part A, it did not appear justifiable to use large quantities of Fraction II in studies on treatment.

Clinical Material

Sixty-one individuals are included in this report. The age range of this group is shown in Table IV. Those selected were as far as possible young children in whom a specified amount of globulin, when considered in relation to the size and weight of the individual, would be relatively more effective. The range of dosage used is also shown in Tables V and VI. The wide variation of dosage is due to lack of accurate knowledge concerning the antibody content of the globulin and the individual choices of physicians who attempted to judge the size of the dose given for treatment by their developing experience with Fraction II in prophylaxis. The difficulties of obtaining a large group of individuals in the home for treatment of measles before the rash has appeared or even during the earliest phases of the rash are far greater than may at first be supposed. The great majority of parents hesitate to call a physician during the earliest stages of any disease. This is particularly true of early measles, with its resemblance to the common cold, for which parents usually delay in calling a physician. In most children, the rash of measles appears before the decision is made.

Results

The individuals treated with the gamma globulin are best divided into two groups, according to presence or absence of rash at the time of injection. The rash by no means appears in a specified number of days from the beginning of the disease, but the sequence of events is fairly

TABLE V

Analysis of results of treatment with gamma globulin

Age	BEFORE RASH—KOPLIK'S SPOTS PRESENT—31: Dosage							RASH PRESENT—30: Dosage									
	5	8	10	12	13	15	20	5	7 and 7.5	8	9	10	12	15	20	25	30 to 35
years				*cc.*								*cc.*					
10/12							1S										
1		1G							1U			1G					
2				1S			2G					1U					
3	1G			1G			1G		1S						1U		
4			1G 1S	1G*			2G					1G					
5			1G	1G			1G					2U		1G	1G 1S		
6	2G** 2U					1U	1G	1U					1G		1S 1U		
7	1U				1S	1U				1G	1S	2U		1S 1U	1S 1U		
8			1U			1S								1U			
9						1S											
10			1U									1U					
11															1U		
12						1G											
Over 12*** (34 years)			1S									1U				1S	2U
Totals	6 3G 3U	1 1G	5 2G 1S 2U	4 3G 1S	1 1S	5 1G 2S 2U	8 7G 1S	1 1U	2 1S 1U	1 1G	1 1S	8 2G 6U	1 1G	4 1G 1S 2U	8 1G 3S 4U		

G—Greatly modified.
S—Slightly modified.
U—Unmodified.

* Developed no rash.
** One developed no rash.
*** Group "Over 12 years" omitted from all totals.

TABLE VI

Summary of results of treatment with gamma globulin **

Ages		Pre-rash	Rash
years			
10/12 to 5	GM	13*	4
	SL. M	3	2
	UN. M	0	5
Totals		16	11
6 to 12	GM	4*	2
	SL. M	3	4
	UN. M	7	9
Totals		14	15

GM—Greatly modified.
SL. M—Slightly modified.
UN. M—Unmodified.
* No rash developed in one.
** Five in group 13 years and over omitted.

uniform, with coryza, conjunctivitis, and Koplik spots preceding the rash by many hours. The lack of such a sequence, at least of the Koplik spots and rash, made early therapy more difficult in a few of those treated. Despite this irregularity, no other landmarks in the field of measles are as stable as these two. This is well demonstrated in Figures 3 and 4 which show data from an epidemic of measles, which occurred during February and March, 1943, in a home for preschool children. The irregularity of appearance of rash following the first signs and symptoms, including Koplik spots, is striking. Because of a special study, all of the children were examined daily or twice daily by the authors, both preceding and throughout the epidemic. The patients were divided, as indicated in Figure 5, according to the presence or absence of rash. The chart also indicates any modification of the disease which it was judged had been obtained by the injected globulin. The classification was similar to the one used in Part A, with the obvious exception that a group with no measles could not

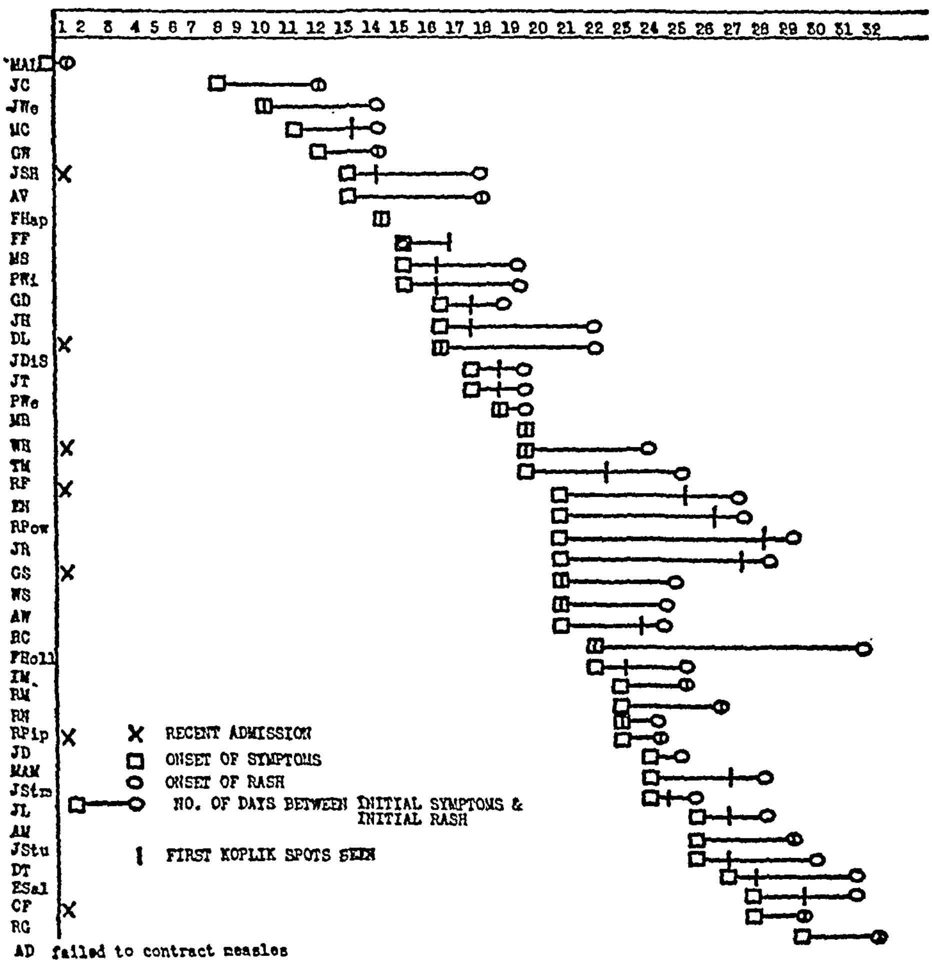

FIG. 3. PROGRESS OF MEASLES EPIDEMIC IN A HOME FOR PRE-SCHOOL CHILDREN, PHILADELPHIA—1943

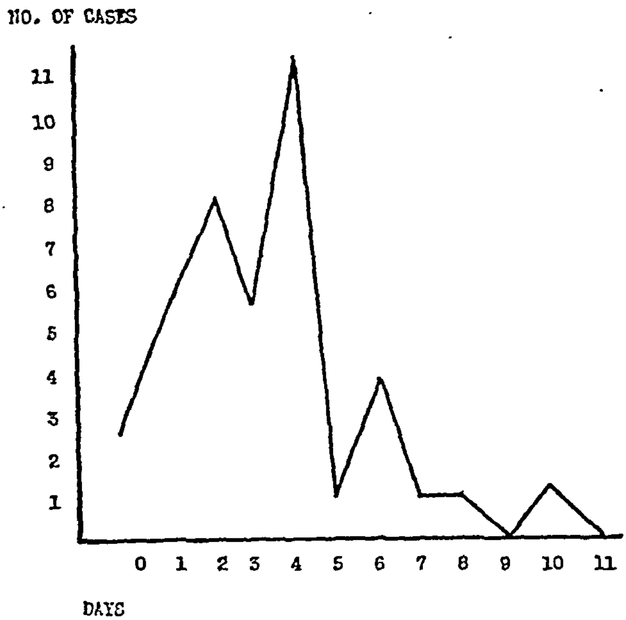

FIG. 4. NUMBER OF DAYS BETWEEN ONSET OF SYMPTONS AND ONSET OF RASH

be included. For a group of this size, judgment as to the value of the globulin in treatment can be more exact than for its value in prophylaxis, because definite alterations in the course and severity of a disease can be noted and even in the early stages of measles some concept of its subsequent severity usually may be obtained. This is well shown by Figure 5 in which the columns appear almost as mirror images. On the other hand, in a study of prophylaxis, alterations in severity can be judged only by the accumulation of a sufficient number of cases for statistical comparison with other large groups. Where feasible, control cases without treatment, occurring at approximately the same time and in the same place, were included, although little importance is attached to obtaining an individual control for each case. As indicated in Part A, greater significance should be attached to over-all severity of the epidemic of which the children are a part than to the severity of the disease in individual controls. The wide variation in susceptibility of different children has been indicated in the curves shown in Figure 2, Part A. Also, it is again worth noting that large families of exposed susceptibilities who have received no human immune bodies will vary greatly in the severity of measles among the individual children. The intimacy of exposure of individual children may be partially responsible for such variations as well as differing susceptibilities, but with such variations in severity of measles, the problem of selecting individual controls remains a difficult one. However, Table IV indicates the comparative severity of the disease in a group of treated children and a group of controls who received no globulin. The controls were either from the same family or had had exposures similar to those of the treated children.

The difference is striking between those 31 individuals treated before the rash appeared, *i.e.*, during the stage of Koplik spots, and 30 treated immediately after the rash appeared. The better results obtained by the earlier use of the globulin are the more striking in consideration of the fact that two children are included in the former group who at no time developed a measles rash, despite a typical onset with many Koplik spots. In both of these children, the fever and other signs and symptoms subsided by crisis. Their occurrence earlier in the course of the study gave greater hope for such therapy in the dosages used than has appeared justified by later findings, however significant these may be. Though only two in number, the result is sufficiently unusual to warrant the conclusion that under the conditions obtaining in these cases, the therapeutic

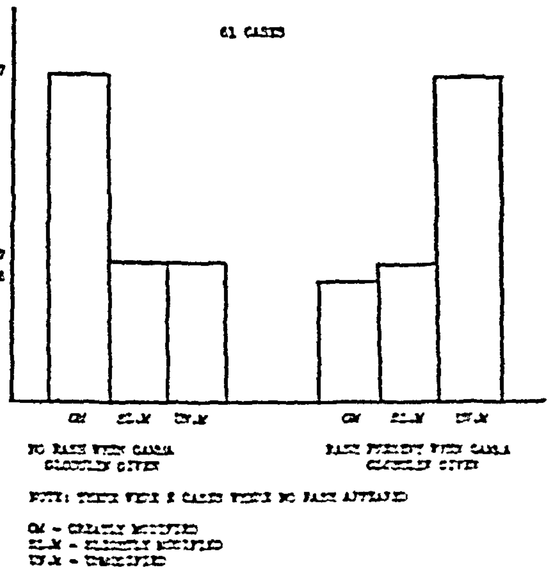

FIG. 5. THERAPEUTIC USE OF GAMMA GLOBULIN IN MEASLES—1943

agent used had curative value. In addition, 5 children in the same group developed very sparse rashes, while the signs and symptoms of early measles rapidly subsided. In contrast and serving essentially as a control to the first group, the majority of the group of individuals treated after the rash appeared gave no evidence of modification of the disease. Nevertheless, a small number in this group showed considerable modification of the disease.

Discussion

The small number of individuals treated does not permit final conclusions concerning the value of this type of therapy, although the data offer some hope that gamma globulin Fraction II (also II + III), or other fractions of a higher order of concentration of measles antibodies in similar or larger amounts, may be of considerable therapeutic value in the early stages of measles. Globulin preparations which could be injected intravenously would be possibly of even greater usefulness, particularly if they were obtained from pools of plasma drawn during the early stages of measles convalescence. The practical application of such therapy is problematical unless more potent preparations, administered intravenously, prove to be highly effective even when the rash has appeared, inasmuch as a physician rarely has the opportunity of treating a child during the stage of Koplik spots with no rash. On the other hand, if such therapy, administered before the appearance of rash, on further study proves to be effective, the proper education of parents concerning measles and their awareness of such therapy may result in the earlier treatment of larger numbers of children.

Passive immunization soon after exposure for purposes of attenuation is preferable to such therapy, but the lack of knowledge of exposure is such a common and unavoidable experience that treatment with human immune bodies by this method, if proved effective, would still be useful.

Summary

The serum gamma globulin, separated and concentrated by chemical fractionation of normal human blood, has been used in studies on the treatment of measles in its early stages. Fraction II + III was used in 8 cases and Fraction II in 53 cases. Dosages ranged from 5 to 35 cc. and ages from 10 months to 34 years.

Of 30 individuals injected after the rash had started, there appeared to be modification of the disease in 13.

Of 31 individuals injected when Koplik spots were present but no rash, there appeared to be modification of the disease in 24. In 2 of these individuals, no rash developed.

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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

XII. THE USE OF CONCENTRATED NORMAL HUMAN SERUM GAMMA GLOBULIN (HUMAN IMMUNE SERUM GLOBULIN) IN THE PREVENTION AND ATTENUATION OF MEASLES[1,2]

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The prevention or modification of measles by means of passive transfer of human antibodies is a well-established procedure. Convalescent measles serum, pooled adult serum, and globulin derived from human placentas have all proved effective. Convalescent serum has probably given the best results, whereas pooled adult serum has been the least satisfactory. The following data, compiled from the literature by McKhann (1), indicate the comparative value of these preparations:

Preparation	Number of Cases	Protection	Attenuation	Failure
		per cent	*per cent*	*per cent*
Convalescent serum	1627	75	17	8
Adult serum	584	56	24	20
Placental extract	2740	64.3	30.4	5.3

This material was unselected, no adjustment being made for dosage, exposure-injection interval, age, or degree of exposure.

The following communication presents the results of studies on the value of Fraction II (Human Immune Serum Globulin) as a prophylactic agent against measles. Fraction II is a serum gamma globulin concentrate derived from pooled normal human plasma by the methods of Cohn, Oncley, Strong, Hughes, and Armstrong (2). Since, as has been demonstrated by Enders (3), the antibodies against certain viruses, such as those of mumps and influenza A, are concentrated many times in this fraction, it was felt that it should constitute an effective prophylactic agent against measles, as originally suggested by Robinson.[3] These studies were begun somewhat later, but carried on at the same time as those of Stokes, Maris, and Gellis (4), reported in the preceding paper.

During the winter and spring of 1942–43, a limited epidemic of measles occurred in and around Boston. This was complicated by a contemporaneous and much more extensive epidemic of German measles. Those cases alone have been included in the survey in which the diagnosis of true measles was unequivocal. Four groups of persons served as subjects during the course of the study: children of families under the care of city physicians, students at several private schools, cases encountered in private practice, and individuals exposed in the wards of a hospital.

CONTROLLED FAMILY STUDY

Most of the primary cases occurred in families in the lower income groups living in crowded quarters, and, accordingly, the exposure of the other children of the family was, *in all but a few* instances, intimate.[4] Each primary case was

[1] This work has been carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[2] This paper is Number 19 in the series "Studies on Plasma Proteins" from the Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross.

[3] See J.A.C.S., 1940, 62, footnote on page 3395.

[4] We wish to acknowledge our indebtedness to the late Dr. Harry Goldman, Deputy Health Commissioner, City of Boston, and his office associates, and to Dr. Joseph Rosenthal and his associates of the District Medical Service, Boston Dispensary, for their invaluable assistance in reporting to us cases of measles with home contacts.

investigated as soon as possible after it was reported to us. If the patient was found to have true measles, those siblings who had no history of measles were used in the study, provided the time relations were appropriate.

The first part of the work comprised an attempt to ascertain, under carefully controlled conditions, the capacity of Fraction II to prevent measles. A single preparation (A66) was used. The dosage was arbitrarily selected as 5.0 cc. for children over 5 years, and 2.5 cc. for children under this age, with the exception of infants between 6 months and 1 year, who were given 2.0 cc. Those under 6 months were presumed to be immune and were not inoculated.

When there were 2 or more susceptible contacts in a family, they were divided into 2 groups composed of persons as nearly alike as possible with respect to age and degree of exposure. Children over 15 years of age were placed in the control group in order to minimize the effects of the greater immunity which has been found to characterize older persons. The globulin was injected intramuscularly in the gluteal region into the members of one group. The other group received no injection and served as a control of the validity of the exposure. The globulin was administered as early as possible after exposure, usually on the fourth or fifth day. The family was visited by one of us at 2- and 3-week intervals after inoculation, or when we had been informed by the family of the first sign of illness in any of the children. At each visit, all children were examined for coryza, Koplik spots, rash, and fever, and inquiries were made of parents and children concerning symptoms and signs which meanwhile might have occurred in any of the contacts.

The second part of the family study consisted of an attempt to attenuate the disease rather than completely to prevent it. The same dosage was used as in the first phase of the study, but the injection of Fraction II was withheld until about the 9th day after exposure. A different preparation (D26) was used in all but 3 cases, in which A35 was used. Return visits were made every 2 days until 3 weeks after injection. The same examinations were carried out and history obtained as in the previous series.

The results of the study are shown in Table I and Figure 1. Table I includes all cases. In 3 families, the adequacy of exposure of the contacts was questioned at the time they were first seen. The fact that subsequently no additional cases developed in the controls or in the inoculated children indicates that either exposure was

TABLE I

Results in controlled family study

(Crude figures)

Aim	Total no. cases	Inoculated	Results: No measles	Results: Mild measles	Results: Typical measles	Uninoculated controls	Results: No measles	Results: Mild measles	Results: Typical measles
Protection	74	45	40 (89 per cent)	5 (11 per cent)	0	29	7 (24 per cent)	2 (7 per cent)	20 (69 per cent)
Attenuation	65	40	27 (67.5 per cent)	12 (30 per cent)	1 (2.5 per cent)	25	4 (16 per cent)	0	21 (84 per cent)
Total	139	85	67 (78.5 per cent)	17 (20 per cent)	1 (1.5 per cent)	54	11 (20 per cent)	2 (4 per cent)	41 (76 per cent)
	(Corrected figures)								
Protection	54	31	26 (84 per cent)	5 (16 per cent)	0	23	1 (4 per cent)	2 (8 per cent)	20 (88 per cent)
Attenuation	54	31	18 (58 per cent)	12 (39 per cent)	1 (3 per cent)	23	2 (9 per cent)	0	21 (91 per cent)
Total	108	62	44 (71 per cent)	17 (27 per cent)	1 (2 per cent)	46	3 (7 per cent)	2 (4 per cent)	41 (89 per cent)

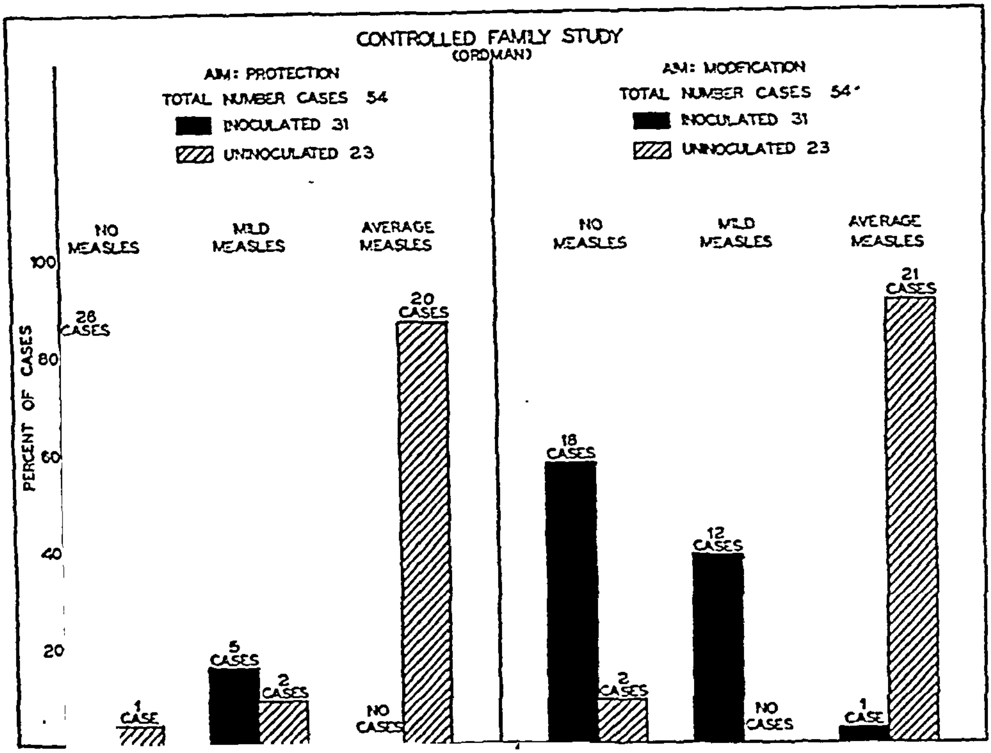

Fig. 1. Controlled Family Study

inadequate or immunity was present. In Table I and Figure 1 are presented the data obtained only in those families in which one or more controls developed measles, or in which one of the inoculated children developed modified or typical measles. It should be emphasized, however, that among 85 susceptibles inoculated with Fraction II, only 1 case of typical measles developed, and, in this instance, the child was accidentally given only 1.5 cc. of preparation D26 on the tenth day after exposure. Of 54 controls, 41 developed typical measles which was of average or severe intensity. Moreover, no complications were observed among the 17 children who contracted measles in a markedly attenuated form after inoculation, whereas, of the controls who got measles, 4 subsequently developed otitis media and 2 developed pneumonia.

EPIDEMICS IN PRIVATE SCHOOLS

An opportunity to observe small or potential epidemics of measles in 3 private schools was afforded us through the courtesy of the school physicians, Dr. J. R. Gallagher of Phillips Academy, Andover, Mass., and Dr. R. K. Byers of Milton Academy (Boy's and Girl's Boarding Schools), Milton, Mass. We are indebted to both of these men for their assistance in following the subsequent course of the patients in these groups.

In the Andover epidemic, 70 presumably susceptible boys, ranging from 13 to 18 years of age, were exposed to 19 cases of measles between January 15, 1943, and February 9, 1943. On February 10, 28 of these boys were inoculated intragluteally, each with 5.0 cc. of A66. Also, on February 10, 7 additional susceptibles, who were already patients in the Infirmary with the questionable diagnosis of measles in the pre-eruptive stage, were given similar inoculations of the globulin. Thirty-five of the boys who denied having had measles were not inoculated, and thus served as controls. The degree of exposure in each case was virtually impossible to ascertain and the exposure-injection interval equally difficult to determine because of the widespread and irregular distribution of the disease throughout the school.

The sudden cessation of this epidemic makes the results hard to interpret. Of the 35 students

inoculated, 9 developed measles within 3 days of injection, suggesting that exposure had occurred some 10 days before inoculation. Five boys developed measles more than 3 days after inoculation, but all these cases were extremely mild in character. Of the 35 controls, 11 developed measles within the first 3 days of our study. Four only came down 3 or more days later, all with disease of at least average severity. Thus, some 21 inoculated students and 20 controls, all of whom were presumably susceptible, failed to develop measles. It was the impression of the school physician that the disease among the inoculated students, even those who developed measles shortly after injection, was less severe in general than among the controls, and that the incidence of complications was less. Only 1 case of otitis media developed in the former group in contrast to 6 cases in the latter group. Moreover, 2 susceptible students, who had been admitted to the Infirmary for illnesses other than measles and had been inadvertently placed in measles wards in close contact with active cases, were inoculated and failed to develop the disease.

In Milton Academy Boy's Boarding School, the study began on February 25, 1943. Two of the students had come down with measles on February 16 and 17, respectively, and a third had developed the disease on February 23. All of them were at large in the school for a day or two, carrying on their normal activities, before the rash was noticed. There were, in the boarding school, 26 presumably susceptible students ranging from 12 to 19 years of age. Twenty-four of these were inoculated intragluteally, each with 5.0 cc. of A66, on February 25, 1943. Two boys, one of whom failed to appear for inoculation and another, who supposedly had had measles previously, became, inadvertently, controls.

Of the 24 inoculated students, 20 did not come down with measles, and 3 developed definitely modified measles. One inoculated boy and both uninoculated controls developed measles of average severity. It is of interest that 7 inoculated students developed symptoms of upper respiratory infection about 2 weeks after injection, but had no other signs of measles. An opportunity to determine, in part, the length of immunity conferred by the globulin was afforded when one of the group who had been inoculated on February 25, developed a typical measles rash on April 16. Subsequently, 11 of the previously inoculated group developed measles within the next month. Of these, 4 exhibited a very mild form of the disease, whereas the remaining 7 had measles of average severity. Six of these were boys who had had symptoms of upper respiratory infection 2 weeks after their original exposure, indicating that they either had had no measles at that time or else the attack was so mild as to have induced no immunity.

At Milton Academy Girl's Boarding School, 2 cases of measles developed on May 2, 1943. Fourteen girls, ranging in age from 15 to 18 years, were presumably susceptible. All of this group had had intimate contact with the primary cases and were injected intragluteally with D26, 5 days after exposure. Seven girls were each inoculated with 5.0 cc. and 7 were given 1.5 cc.

Of the 7 girls who had received 5.0 cc. of globulin each, 1 developed no disease, 5 had very mild measles, and 1 had an average case. Of the 7 who received 1.5 cc. of globulin, 4 were apparently protected, 1 developed mild measles, and the other 2 had average severe cases. The higher incidence of disease among those who received the larger dose can probably be accounted for by the small number of cases in each group and the apparent inadequate selection of cases for each group.

Table II includes the total uncorrected figures for all schools, and shows the results obtained when correction is made for the sudden cessation of the Andover epidemic by eliminating that group of cases.

CASES ENCOUNTERED IN PRIVATE PRACTICE

A number of physicians in Boston and its vicinity were given certain of the preparations for use in their private practices. Their cooperation, interest, and careful observation of their patients have been very helpful in this study. Most of these physicians had had considerable previous experience with the use of placental extract. One hundred and ninety-six supposedly susceptible individuals were inoculated with one or another preparation of Fraction II. The dosage and exposure-injection interval were left to the discretion of the individual physician, so that these factors, as well as degree of exposure, age

TABLE II

Results in school epidemics

(Crude figures)

Total no. cases	Inoculated	Results						Uninoculated controls	Results				
		No measles	Per cent	Mild measles	Per cent	Typical measles	Per cent		No measles	Per cent	Mild measles	Typical measles	Per cent
110	73	46	65	14	19	13	16	37	24	65	0	13	35
(Corrected figures)													
40	38	25	66	9	23	4	11	2	0		0	2	100

of contact, and use of controls varied considerably. A standard report form was returned by the physician after a period of from 3 to 4 weeks. Only 8 uninoculated controls were noted in this group of reports.

TABLE III

Results in cases in private practice

(Crude figures)

No. cases inoculated	No measles	Per cent	Mild measles	Per cent	Typical measles	Per cent
196	99	51	87	45	10	4
(Corrected figures)						
164	71	43	83	50	10	7

The data presented in Table III show that the percentage of individuals protected was smaller than in the controlled family group. The total number of cases, however, in which it can be fairly stated that the globulin exerted a modifying effect on the disease represents 96 per cent of the whole number of the presumably susceptible children who were inoculated within 11 days after their initial exposure. When those with less than intimate exposure, those who might in all probability have been immune before injection, those on whom data was inadequate, or those who were inoculated after the ninth post-exposure day are excluded, 93 per cent of the presumably susceptible children either did not contract measles or had the disease in a mild form.

INDIVIDUALS EXPOSED IN A HOSPITAL

During the course of our study, a number of cases of measles developed among patients on the wards of a hospital. In each case, most of the presumably susceptible children on the ward where the disease occurred, regardless of intimacy of contact with the primary case, were inoculated intragluteally with one of the preparations of Fraction II, the dosage being set at 2.5 cc. for children under 5 years and at 5.0 cc. for those over that age. The exposure-injection interval varied from 0 to 11 days, although in most cases globulin was given within 5 days of exposure.

Of the 82 children inoculated, 77 appeared to be protected and 5 had definitely modified measles. However, among 18 presumably susceptible uninoculated children, only 2 known cases of measles developed. Thus, it is apparent that the degree of exposure encountered in a well-run hospital ward is minimal and evaluation of the efficacy of an anti-measles preparation of this sort under these conditions is impossible without adequate controls and a satisfactorily high morbidity rate in the control group.[4]

DISCUSSION AND SUMMARY

The evidence derived from the controlled study in families indicates that Fraction II is a good source of measles antibody. Moreover, this evidence is supported by the results of the trials of the material which were carried out under other conditions. From the standpoint of protection, the product appears to be at least as effective as convalescent serum or placental extract, and probably superior to normal human serum in

[4] By the time of publication, Fraction II had been given to 350 susceptible children exposed in 35 separate outbreaks of measles on the wards of various hospitals. Of these, 95 were not followed, 241 did not develop measles, 13 developed mild measles, and 1 typical measles.

amounts usually administered (1, 5, 6). In view of the finding of Enders (3), that the concentrations of antibodies in Fraction II reacting with certain viruses, bacteria, and bacterial toxins are from 10 to 40 times as great as those in the plasma from which the particular preparation was derived, it is not surprising that measles antibodies should be present in adequate concentration. This concentration of antibody is made apparent by the dose apparently necessary for protection. Although no set figures can be arbitrarily stated, the consensus of most authors appears to be that from 5 cc. to 20 cc. of convalescent serum, from 10 cc. to 80 cc. of pooled adult serum, and from 1 cc. to 4 cc. of placental extract are necessary to afford complete protection in the large majority of cases.

Dosage. Although we have not accurately determined the minimum effective dose of Fraction II, it is apparent that 5 cc. was adequate for protection in most of our cases, and in the smaller children, 2.5 cc. gave equally good results. It is also apparent that the exposure-injection interval plays a rôle in determining whether protection or attenuation is to result from treatment. Approximately the same proportion of individuals was protected if the injection was given in any one of the first 5 days, but from the sixth day onward, the percentage of attenuated cases and failures increased. In Figure 2 are plotted the doses per pound of body weight as ordinates, and as abscissae, the days which elapsed between first exposure and injection. This chart includes data from all of our groups of cases, but no cases

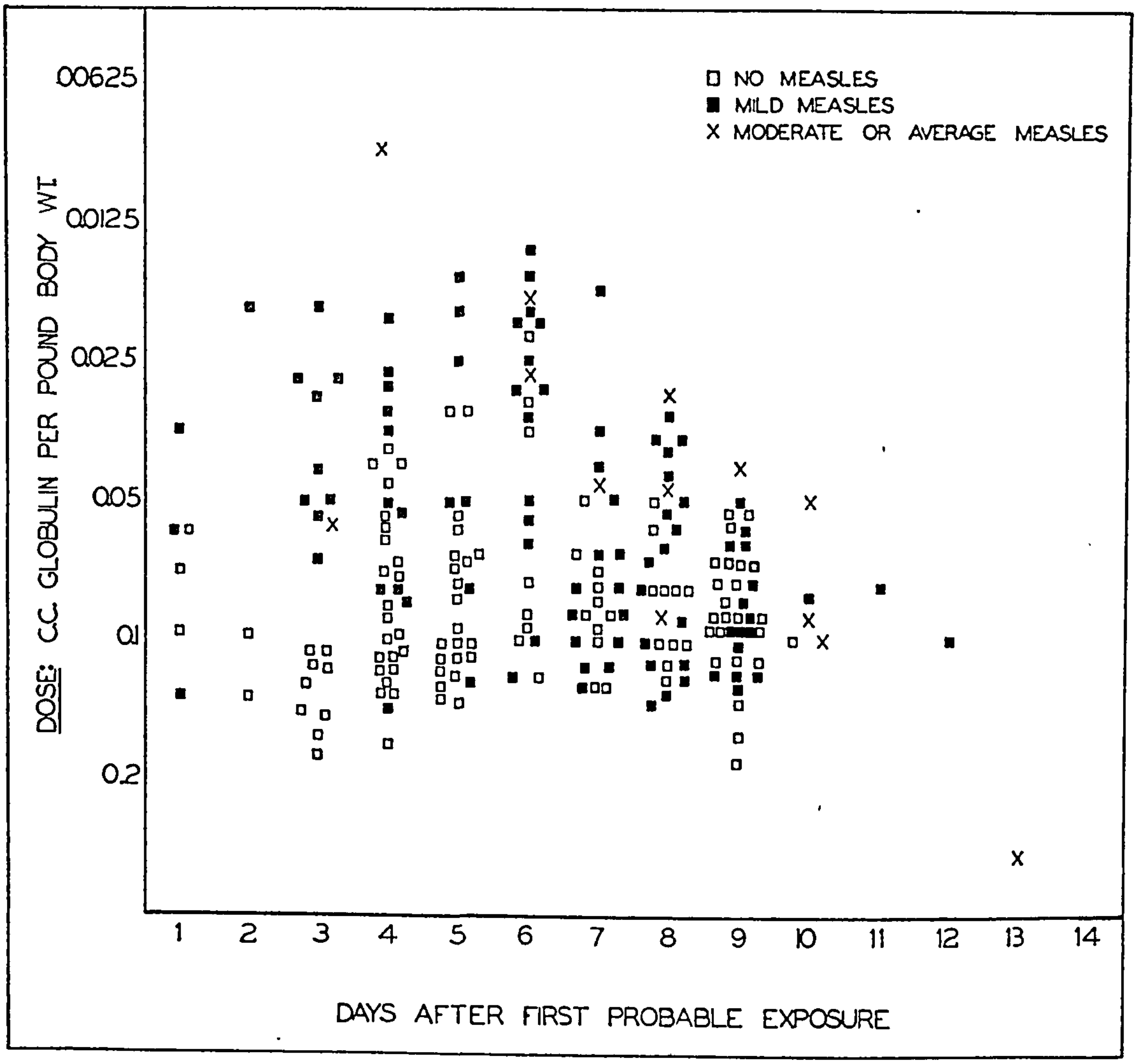

FIG. 2. RELATION OF RESULTS OBTAINED FOLLOWING INJECTION OF NORMAL HUMAN SERUM GAMMA GLOBULIN TO DOSAGE ON A WEIGHT BASIS AND TO EXPOSURE-INJECTION INTERVAL.
Data obtained from 222 cases with intimate exposure and adequate follow-up.

have been included in which there was not intimate exposure for a reasonable period of time, reasonable evidence of susceptibility, or adequate data upon the results of injection. The great majority of the 222 cases recorded in Figure 2 were children under 10 years of age. Whether results obtained in this age group may be carried over to adolescents and adults by calculating the dose on a weight basis is not definitely known. It is apparent from the figure that, with 3 exceptions, all those individuals receiving 0.1 cc. per pound of body weight or more within the first 5 days after exposure were protected. When the dose was less than 0.1 cc. per pound of body weight or the interval after exposure longer than 5 days, there was a decrease in the number of cases protected and an increase in the number of cases developing mild (modified) measles. A dose of approximately 0.025 cc. per pound in the first 5 days resulted in the development of mild measles in most cases, but the numbers are too small for definitive conclusions. Finally, when very small doses were employed or the exposure-injection interval prolonged beyond 9 days, no effect on the disease was usually obtained. Further investigation is planned which, it is hoped, will more accurately establish the relationship between time and dosage.[5]

Duration of immunity. The family study yielded some information on the duration of the passive immunity produced by Fraction II, since in all families where the controls came down with measles, the inoculated children were thus subjected to a new and intimate exposure within 5 to 14 days after injection. In no case did the inoculated and re-exposed children develop recognizable measles. This suggests that an effective immunity lasts for at least 2 weeks after the injection of a dose of the size used. It is of interest that one nephrotic boy, who received what should have been a protective dose, developed typical measles, suggesting that the antibody may have been rapidly lost due to the proteinuria. The results in the Milton Academy Boy's School suggest that the immunity produced by the injection of 5 cc. does not last for 7 to 10 weeks, since the majority of previously inoculated boys, re-exposed at this time, developed average measles. McKhann (1) has emphasized the prolongation of the incubation period in cases of modified measles, but in most of our modified cases, the rash appeared 10 to 15 days after that of the primary case. In a few patients, symptoms were observed only during the third week, so that a 3-week period of observation for inoculated cases is probably advisable.

Reactions. In spite of the established value of convalescent serum, placental extract, and adult serum, certain disadvantages are associated with each preparation. Convalescent serum is available in relatively limited quantities. Normal pooled adult serum is readily available, but its low potency necessitates the injection of large volumes, which is undesirable, particularly in small children. However, 2- to 4-fold concentration of serum has been achieved by the process of desiccation from the frozen state, the resulting dried powder being reconstituted with a smaller volume of water than was present in the original material. Such concentrated serum has proved both safe and effective for the prophylaxis of measles (7). Placental extract, although available in quantities and requiring only a small inoculum, causes local and systemic reactions of varying severity in a considerable number of inoculated individuals.

Fraction II, in contrast, offers definite advantages. It is readily produced from an abundant source of supply. In small doses, it has proved to be as effective as the best of the standard preparations. No severe reactions have been observed in the several hundred individuals inoculated with it. In less than 5 per cent of these, mild reactions occurred. With a single exception, the reactions consisted of a slight feeling of stiffness in the muscle injected or a little local erythema and induration. In one case, the individual had a rise in temperature to 102° F. 2 days after inoculation but no other systemic or local manifestation. Whether or not this febrile reaction was due to the globulin cannot be stated.

So far no untoward sequelae have been observed, following the use of Fraction II intra-

[5] Studies carried out in 1944 in a much larger series of cases and with a large number of preparations have confirmed the validity of these recommendations as to dosage. It is now clear that in order to achieve protection or attenuation the physician should vary the dose rather than the interval between exposure and injection.

muscularly, but the possibility that viruses present in the pooled plasma from which Fraction II is obtained might be carried over in the process of separation cannot be entirely neglected. Homologous serum jaundice, which has been described following the injection of human serum in yellow fever immunization (8) and in passive protection against measles and mumps (9), has an incubation period of 1 to 4 months. As many cases as could be followed were visited or questioned by letter 3 to 6 months after inoculation. Of 400 cases so followed, one case of typical catarrhal jaundice was noted just 3 months after an injection of 5 cc. of globulin. That this was pure coincidence is suggested by the fact that although 74 others are known to have received this preparation, no other cases of jaundice have been reported to us. It is hoped that answers to these and other problems will be forthcoming as a result of work now in progress.

The necessity for observing certain precautions in a survey of this sort is brought out very clearly in several of our studies. One must eliminate the possibilities, in so far as possible, of insufficient exposure, previous unrecognized clinical attacks, and unrecognized modified attacks at the time of investigation. In a number of infectious diseases, laboratory tests exist which can, with some certainty, eliminate various of these factors, while others may often be excluded by careful histories and physical examinations. In the case of measles, we are, as yet, entirely dependent on the latter methods and an evaluation of the results of a study must be carried out with the factors mentioned above clearly in mind.

The natural secondary attack rate of measles in an urban community has been established at about 75 per cent for all ages, rising to 85 to 90 per cent for children between 1 and 10 years of age and dropping sharply after 10 to between 15 and 40 per cent (10). In our investigation, by far the greatest number of inoculated individuals were in the 1- to 10-year age group.

In addition to age, intensity of exposure is of great importance in determining the secondary attack rate. In our hospital ward cases, for example, exposure was apparently entirely inadequate and no fair assumption may be made therefrom. It has been shown (6, 10) that prolongation or repetition of exposures is of no significance, providing the original exposure is sufficiently intimate. One cannot assume that exposures in schools, hospitals, or on the playground are adequate. On the other hand, exposures within the family, of children within the same age group, are usually intimate. We have included in our corrected tables (excluding those in the school epidemics) only such cases, together with a few others where adequate proof of close exposure existed. Even under such conditions, there may be some doubt as to the adequacy of the exposure or about other factors such as pre-existing immunity. Accordingly, in order to obtain results of the greatest reliability possible, it is wise to have controls such as were demanded in our family study. The corrected tables in that study include data only from those cases in which one or more controls came down with measles or where the inoculated individual developed the disease.

Stillerman, Marks, and Thalhimer (5) have emphasized the necessity of careful observation of inoculated persons in order to detect the mildest cases of measles, which might escape any but trained and experienced observers. Undoubtedly, a number of individuals who might ordinarily be classified as having been completely protected exhibit, at one time or another, one or more of the stigmata of measles in very mild or bizarre form, and unless daily observations are made of each person, these might readily be missed. Their total of 77 per cent complete protection or very mild measles corresponds closely, as they note, with the figures given by most authors for complete protection alone with convalescent serum and with the 71 per cent protection obtained by us in our family survey. It is possible that the percentage of protection recorded by us may include some cases of very mild unrecognized measles.

CONCLUSIONS

1. The human serum gamma globulin (Fraction II), separated and concentrated by chemical fractionation of normal human blood, is a very satisfactory prophylactic agent against measles.

2. A controlled group of cases with exposure within the family afforded a rate of 71 per cent protection, 27 per cent modification and only 2 per cent failure, among 62 inoculated children.

Of 46 uninoculated controls, only 7 per cent failed to contract measles, while 4 per cent got mild measles, and 89 per cent developed measles of average severity.

3. In children, an intramuscular dose of 0.1 cc. per pound of body weight within the first 5 days after exposure appears to be adequate for complete protection in a large majority of cases, with the present methods of preparation. In order to produce attenuation of the disease, it would seem that about one quarter this dose should be administered during the first 5 days after exposure. After this, somewhat larger doses may be necessary.

4. No significant untoward reactions were observed in any of the inoculated cases.

5. The importance of adequate controls, intimate exposure, and careful observation of inoculated individuals in evaluating the efficacy of a prophylactic agent against measles is emphasized.

This work was carried out with the technical assistance of Miss Virginia S. Poole, B.A.

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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

XIII. THE SEPARATION AND CONCENTRATION OF ISOHEMAGGLUTININS FROM GROUP-SPECIFIC HUMAN PLASMA [1,2]

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At the beginning of the twentieth century, Landsteiner (1, 2) reported the presence of normal isohemagglutinins in human serums and their corresponding agglutinogens in the red cells. On the basis of these observations, normal human blood is classified into 4 groups, *i.e.*, Groups O, A, B, and AB. Continued research has served to confirm Landsteiner's observations and postulates. Landsteiner found further that an isohemagglutinin and its homologous agglutinogen could not exist together in the same blood, and that their relationships are reciprocal; *i.e.*, the presence of one indicates the absence of the other.

With the advent of whole blood transfusions as an important therapeutic procedure, it became necessary to determine the blood groups of both donors and recipients prior to transfusion. A number of methods have been described for assigning an unknown blood to one of the 4 blood groups. In practice, they involve the collection and preservation of 2 grouping serums, containing, respectively, avid Anti-A and Anti-B agglutinins, preferably in high titer. The isohemagglutinin serums are mixed with the cells of the unknown blood under appropiate conditions which will give prompt true agglutination if A and/or B cells are present. The cells of a Group O blood are not agglutinated by either Anti-A or Anti-B serum; the cells of a group A blood are agglutinated by the Anti-A but not by the Anti-B serum; the cells of a Group B blood are agglutinated by an Anti-B serum but not by an Anti-A serum; and the cells of an AB blood are agglutinated by both Anti-A and Anti-B serums. It is not within the scope of this paper to present blood grouping methods in detail and the reader is referred to the many excellent blood grouping manuals for further information (3, 4).

It is, however, apparent that specific avid high-titered grouping serums are necessary for accurate blood grouping. The collection of high-titered serums from special donors has been the major means of procuring such serums in the past. Recently, Wiener (5) has been able to increase both the titer and the avidity of the plasma isohemagglutinins by intravenous injection of dried human plasma into special donors. Witebsky (6) has also been able to accomplish the same effect by the injection of small doses of A and B specific substances into Group A and B individuals. Immunization of rabbits and absorption with group-specific human red blood cells has also led to the production of satisfactory grouping serums.

Recently, the necessity arose at the Army Medical School to obtain large quantities of potent grouping serum. The difficulties in obtaining donors with sufficiently high-titered isohemagglutinins in their serum made it necessary to investigate the possibility that chemical or physical concentrations of plasma from random donors of proper group might offer a means of procuring a sufficient quantity of uniform high-titered grouping material.

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[1] This work has been carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[2] This paper is Number 20 in the series "Studies on Plasma Proteins" from Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross.

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Thalhimer and Myron (7) have shown that the isohemagglutinins could be separated and concentrated from serum or plasma by ammonium sulfate precipitation. Pillemer (8) later showed that the concentration of the isohemagglutinins from serum could be very easily accomplished by the use of methanol as a precipitant. The globulins separated by this method exhibited marked avidity for incompatible red cells and showed a 4- to 8-fold isohemagglutinin concentration over serum.

As reported earlier in this series of papers, among the variety of plasma components with diverse physiological and immunological functions, that are available as by-products of the human serum albumin program, are included the isohemagglutinins (9). Boyd, working in the Department of Physical Chemistry, Harvard Medical School, has noted that Fraction II + III, obtained as a by-product in the fractionation of human plasma for production of serum albumin, has isohemagglutinin activity (10). Since large amounts of Fraction II + III would be available as a by-product, it was thought advisable to extend the studies which had been started at the Army Medical School with the possibility of obtaining high-titered grouping material from Fraction II + III. The following report is a summary of the results obtained with products prepared at the Harvard Fractionation Laboratory. It will be seen that a potent grouping reagent, which can be distributed as a white powder, has been prepared routinely as a by-product of the human serum albumin program.[6]

COLLECTIONS AND ASSAY OF GROUPING SERIES

The initial step in the preparation of an isohemagglutinin-containing fraction from human plasma consists in the collection of blood from donors of a single group, either A or B, for a given pool. While each pool has thus been derived from donors of the same blood group, no attempt has been made to select high-titered plasmas. Of course, initial titration of each individual plasma, in order to exclude low-titered plasmas, will lead to a much more potent grouping material than is currently obtained. The titers of the pooled plasmas processed have varied from 1:32 to 1:128 and from this material, final products having titers from 1:512 to 1:2048 have been obtained.

Two methods of assay have been employed in this laboratory in the determination of the isohemagglutinin activity of the plasma pools and the final isohemagglutinin globulins. These will be briefly mentioned here.

SPEED AND INTENSITY OF AGGLUTINATION (AVIDITY)

Group A Serum (Anti-B). One drop (0.05 ml.) of serum or isohemagglutinin containing globulin solution is mixed on a slide with a drop (0.05 ml.) of a fresh group B cell suspension (5 per cent). The slide is constantly rocked, and the time necessary for beginning visible macroscopic agglutination as well as the time required for complete agglutination is recorded.

Group B Serum (Anti-A). In general, this is the same as for the Anti-B serums except that the avidity is tested against A_1, A_2, and A_2B cells.

ANTIBODY CONTENT (TEST TUBE TITER)

The plasma or globulin preparation is geometrically diluted in a series of test tubes so that each tube contains 0.2 ml. of solution in dilutions 1:1, 1:2, 1:4, 1:8, 1:16, and on in the same progression up to 1:4096. Two-tenths of a ml. of a 2 per cent suspension of incompatible red cell suspension (Group A in the case of testing agent Anti-A and Group B in the case of an Anti-B serum) is added to each tube. The contents of the tubes are well agitated and the tubes then centrifuged at 1000 R.P.M. for 1½ minutes. The packed cells in each tube are gently shaken up from the bottom of the test tube and the degree of agglutination recorded: 4 plus = one large clump; 3 plus = 2 to 4 medium size clumps;

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[6] As will be shown in a subsequent paper of this series, eight preparations of isohemagglutinin globulin prepared at the Harvard Pilot Plant have been evaluated at the request of the Subcommittee on Blood Substitutes of the National Research Council by Dr. Elmer L. DeGowin and a panel of investigators chosen by Dr. DeGowin. The reports from this panel were invaluable in that the preparative group were mainly guided by their appraisals, and are summarized in the following paper in this series (11).

2 plus = several medium size and small clumps; one plus = several very small clumps.

PROPERTIES OF THE ISOHEMAGGLUTINATING GLOBULINS

A purified euglobulin fraction of plasma, in which the isohemagglutinin activity is mainly concentrated and which may be dispensed in a dried stable state, has been prepared routinely at the Harvard Pilot Plant. The dried powder dissolves readily upon the addition of proper amounts of distilled water and is highly active for blood grouping purposes.

The separated isohemagglutinin containing globulins represents about 5 per cent of the proteins present in plasma. Electrophoretically, this protein fraction is composed mainly of gamma and beta globulins. Work is in progress on the further purification of this fraction in order to establish the chemical identity of the isohemagglutinins. It should be pointed out here that as yet no chemical or physical differences have been encountered between the Anti-A and Anti-B isohemagglutinins, respectively.

TABLE I

The concentration of isohemagglutinin globulins over plasma

Run	Plasma			Fraction III-1		
	Avidity		Test tube titer	Avidity		Test tube titer
	B[a]	C[b]		B[a]	C[b]	
	seconds			*seconds*		
89A	30	150	1 : 32	5	30	1 : 512
90B	15	90	1 : 64	5 15	30-A_1[c] 60-A_2	1 : 1024 1 : 256
91A	30	150	1 : 64	5	30	1 : 1024
94B	15	90	1 : 64	5 15	30-A_1 60-A_2	1 : 1024 1 : 256
92A	30	150	1 : 32	5	30	1 : 512
9094B	15	90	1 : 64	5 15	30-A_1 60-A_2	1 : 1024 1 : 128
9193A	30	150	1 : 32	5	30	1 : 512
104B	30	90	1 : 64	5 15	30-A_1 60-A_2	1 : 1024 1 : 256

[a] Beginning agglutination.
[b] Complete agglutination.
[c] Indicates type of A cell employed.

It will be seen from Table I that the isohemagglutinins in plasma have been concentrated approximately 16 times over plasma. Since approximately 50 to 60 ml. of this material are obtained per liter of plasma, this means that almost quantitative yields have been achieved.

Speed of reaction (avidity) between red cells and the isohemagglutinins

On a glass slide the products will produce macroscopic agglutination in 5 to 15 seconds and complete agglutination of incompatible cells in 30 to 60 seconds.

Specificity of reaction

No rouleaux or false agglutination with compatible cells have been observed in any of the preparations thus far carried out.

Stability

The material is stable under ordinary conditions. Tests have run for 10 days at 50° C., 53 days at 37° C., and 4 months at room temperature (+25° C.). The outcome of further tests will be needed to establish definitely the criteria for stability.

Reproducibility

A uniform product has been obtained from all pooled type-specific plasma processed at the Harvard Pilot Plant. There is reason to believe that this will continue to be true on large scale production since the material can be concentrated or adjusted to the desired specified activity. Furthermore, preliminary selection of only high-titered plasma will doubtless guarantee an even more satisfactory product.[7]

Subgroups specificity

Due to the large number of individual plasmas in each pool, satisfactory agglutination of the

[7] Upon the request of the National Research Council, two preparations of Anti-A and Anti-B isohemagglutinin globulin, respectively, were prepared by the Harvard Pilot Plant to serve as reference standards in evaluating subsequent preparations of isohemagglutinins. The Anti-A has a titer of 1 : 1024 and the Anti-B of 1 : 512. These standards meet the requirements and specifications of the Armed Forces and were approved by DeGowin and his group.

weak subgroup A cells should be achieved. If deemed necessary, preliminary selection of B plasmas will assure this.

Ease of manipulation of grouping tests

Since the material can be dispensed in a dried state which can readily be reconstituted into a stable solution, definite concentrations of it can be added to the red cells, thereby achieving a high degree of uniformity in grouping tests.

Since some service laboratories are not equipped or not readily prepared to do either test-tube or microscopic blood grouping tests, the ready availability of a stable, specific, rapid reagent for macroscopic use on slides offers decided advantage.

Physical properties

The grouping reagent prepared by these methods also lends itself well for blood grouping purposes because of its suitable viscosity and clarity.

SUMMARY

A specific, stable, and highly active isohemagglutinating material has been prepared as a by-product of the human serum albumin program. A concentration of isohemagglutinating activity over plasma of 16 times has been achieved. The properties of this material are described.

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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

XIV. APPRAISAL OF ISOHEMAGGLUTININ ACTIVITY [1]

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(Received for publication February 17, 1944)

The development of the by-products, from the preparation of human serum albumin for the Armed Forces (1), made available large quantities of globulins containing isohemagglutinins. It was found possible to produce material rich in isohemagglutinins from pools of human group-specific plasma, derived from bloods unselected for agglutinin titer. The method of preparation has been reported by Pillemer and co-workers (2). It became the problem of the Subcommittee on Blood Substitutes of the National Research Council to evaluate the product. A group of consultants,[2] qualified in the field of blood grouping, collaborated in testing various lots of concentrated isohemagglutinin preparations made at the Pilot Plant, Department of Physical Chemistry, Harvard Medical School. Samples from 14 lots of material were distributed to the investigators for study. The first 6 lots were in the liquid form while the remaining 8 were converted to the dry state and sealed *in vacuo*.

The consultants all agreed that the 14 preparations submitted for evaluation were sufficiently potent to be acceptable as grouping sera. These conclusions were based on each man's experience in testing the various lots by his own method and comparing the reactivity with that of other grouping sera which he had previously found satisfactory for routine use.

A secondary result of this project led to some rather surprising conclusions. Many writers on the technic of blood grouping have published minimum titration values for desirable grouping sera. It has been tacitly assumed that these numerical results could be duplicated by other workers without much regard for the details of the method employed. This study afforded the opportunity to compare titration values obtained by 11 experts on the same material. Each consultant reported the maximum dilution of serum (uncorrected for added cell suspension) in which agglutination of test erythrocytes occurred. He also noted the appearance time of macroscopic agglutination in a serum-cell mixture on a manually agitated slide and the time at which agglutination was completed. The results showed the wide ranges indicated in Table I.

It was evident from an analysis of the results of each worker that although his values were not comparable with those of another on the same serum, by employing his own method, fairly consistent agreement could be obtained with the others as to the relative potency of various lots of isohemagglutinins. In a conference of the consultants, the variables in the methods of evaluation of the sera were discussed. Aside from the details of mixing serum and cells, it was agreed that the chief variables were the different criteria of the end-point of agglutination, the variations in sensitivity of the erythrocytes derived from different sources, and the differences in concentrations of the cell suspensions employed in determining the agglutination time. Some methods involved the detection of minimal agglutination with the microscope and others

[1] The products of plasma fractionation employed in this work were developed from blood, collected by the American Red Cross, by the Department of Physical Chemistry, Harvard Medical School, Boston, Massachusetts, under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[2] Dr. W. L. Boyd, Boston University; Dr. L. W. Diggs, University of Tennessee; Dr. Paul I. Hoxworth, Cincinnati General Hospital; Dr. Karl Landsteiner, Rockefeller Institute (Dr. Landsteiner died before the completion of this work); Dr. Philip Levine, Newark Beth Israel Hospital; Dr. R. O. Muether, St. Louis University; Dr. Max M. Strumia, Bryn Mawr Hospital; Dr. William Thalhimer, Manhattan Convalescent Serum Laboratory; Dr. Alexander S. Wiener, Office of the Chief Medical Examiner of New York City; Dr. Ernest Witebsky, Buffalo General Hospital; Dr. Elmer L. DeGowin, State University of Iowa.

TABLE I

Results obtained by various consultants on titration of isohemagglutinins

Serum lot number	Group of test cells	Titrations in serum dilutions, ranges	Agglutination time in seconds,* ranges
81A	B	1 : 32 to 1 : 1000	10–40 to 15–60
81B	A_1 A_2	1 : 64 to 1 : 1000 1 : 20 to unreported	10–40 to 15–60
85A	B	1 : 20 to 1 : 200	10–30 to 35–110
86B	A_1 A_2	1 : 40 to 1 : 532 1 : 10 to unreported	7–40 to 43–98
87A	B	1 : 16 to 1 : 800	11–50 to 30–300
88B	A_1 A_2	1 : 64 to 1 : 800 1 : 20 to 1 : 80	7–24 to 15–600
89A	B	1 : 30 to 1 : 640	4–19 to 30–120
90B	A_1 A_2	1 : 120 to 1 : 640 1 : 30 to 1 : 128	3–8 to 20–60 8–53 to 30–180
91A	B	1 : 32 to 1 : 160	7–18 to 25–120
94B	A_1 A_2	1 : 120 to 1 : 640 1 : 32 to 1 : 64	5–15 to 20–60 15–30 to 60–180
92A	B	1 : 40 to 1 : 320	4–12 to 15–120
9094B	A_1 A_2	1 : 120 to 1 : 320 1 : 16 to 1 : 128	3–14 to 15–60 8–32 to 40–180
9193A	B	1 : 32 to 1 : 160	8–24 to 17–52
104B	A_1 A_2	1 : 16 to 1 : 320 1 : 8 to 1 : 80	3–30 to 25–45 16–75 to 50–

* Agglutination time is expressed as: seconds required for appearance of macroscopic agglutination—seconds for agglutination to be completed, e.g., 7–30.

employed the criterion of the appearance of macroscopic clumps of red cells. This factor is readily capable of standardization. Careful attention to the concentration of red cell suspensions in the determination of agglutination time would lead to more nearly reproducible results. Hitherto, there has been no satisfactory method of testing the sensitivity of the erythrocytes employed in the tests. There is agreement that the cells ought to be freshly collected. Each worker must therefore rely on sources of test cells convenient to his laboratory. Ordinarily, this makes difficult the comparison of the results of 2 workers.

The device of employing a "reference serum" was finally adopted. Lots 9193A and 104B were made in quantity at the Harvard pilot plant. These preparations were attested by the chemists as being representative of the chemical methods employed. The dried material was placed in glass ampules and sealed *in vacuo*, so that 5 cc. of the reconstituted material was contained in each package. The lots were judged acceptable as grouping sera by all the consultants who tested them. It was understood that the reference sera represent neither the maximum, minimum, nor optimum potency but that they contain arbitrary concentrations of isohemagglutinins which are acceptable for use as grouping sera. They can be employed in either of 2 ways. The sensitivity of the agglutinogens in the test erythrocytes may be determined by titration against the reference serum. This would seem to remove the last serious obstacle to an accurate standardization of the method of titration of grouping sera. In lieu of this, the relative potency of a grouping serum may be determined with respect to the reference serum by testing both simultaneously against the same suspension of red cells, the same methods being employed for each serum. The investigator may then state the strength of the unknown serum in terms of the potency of the reference serum with respect to the test cells employed. If the unknown is approximately twice the strength of the reference material, it may be expressed as 2R; if half as strong, as 0.5R.

SUMMARY

All 14 preparations containing isohemagglutinins, concentrated from pooled human plasma of appropriate blood group, but unselected for agglutinin titer, made by the method of Pillemer and co-workers, were found acceptable as grouping sera by a panel of consultants, working under the auspices of the Subcommittee on Blood Substitutes of the National Research Council.

When various experienced workers tested the same preparation of isohemagglutinins for titer and agglutination time, it was found that numerical values obtained by one worker could not be compared with those of another without a degree of standardization of technic which does not at present exist in this field. Some of the variation in results could be attributed to different criteria employed in judging the end-point

of agglutination. Another factor was the variation in sensitivity of test red cells, derived from different sources.

A lot of material containing anti-A agglutinins and one containing anti-B activity were accepted by the consultants as reference sera. These were packaged to insure maximum stability. They may be employed to determine the sensitivity of test erythrocytes or in comparison with sera of unknown potency in simultaneous parallel tests.

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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

XV. THE PROTEINS CONCERNED IN THE BLOOD COAGULATION MECHANISM[1,2]

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(Received for publication February 17, 1944)

The profound biological significance of the clotting of blood needs no emphasis here; the vast number of studies of the process which have been carried out testify sufficiently to its importance, and also show clearly the incompleteness of our knowledge of the underlying mechanisms.[4]

The properties of fibrinogen solutions and of fibrin clots may be profoundly modified in many ways by suitable variations in the conditions of preparation. Thus a wide variety of products is obtainable. In the subsequent papers of this series, the properties and uses of several of these, namely, fibrin clots, fibrinogen plastics, fibrin films, and foams made of fibrinogen and thrombin, will be considered. Here, we shall discuss the properties of certain preparations of protein fractions from human plasma, which may be obtained in active and stable form and which possess specific action on the various aspects of the blood clotting mechanism.

The fundamental feature of this mechanism is the transformation of a solution of the protein fibrinogen into the rigid insoluble fibrin clot. This transformation does not occur spontaneously in solutions of sufficient purity, but is normally brought about by the action of thrombin on fibrinogen. Thrombin, of course, does not occur in significant amounts in normal plasma, but is derived from its precursor, prothrombin. The transformation of prothrombin to thrombin commonly requires the presence of calcium ions, and of some member of the class of activating agents known as thromboplastins. Such a reaction may occur to a very limited extent in circulating blood; but the traces of thrombin which may be so formed are rapidly neutralized by certain constituents of blood plasma which have been termed antithrombins.

A fibrin clot, once formed, disintegrates, even under rigidly sterile conditions, in a period which may vary from hours (or even minutes) to weeks. There is now decisive evidence that this disintegration is the result of enzyme action; the enzyme concerned, commonly designated as fibrinolysin, is apparently a proteolytic substance closely allied to trypsin. Some indications suggest that this enzyme may play an important part in the earlier stages of the coagulation mechanism.

Thus the following discussion will deal with 3 groups of materials:

1. Substances which form the structural basis of the clot: fibrinogen and fibrin.
2. Substances concerned with inducing the process of clot formation: prothrombin, thromboplastin and thrombin, and a special type of "globulin substance" which promotes the clotting of hemophilic blood.
3. Substances which produce dissolution of the fibrin clot: fibrinolysins.

FIBRINOGEN AND FIBRIN

Fibrinogen may be determined in plasma or plasma fractions by electrophoretic studies, in

[1] This work has been carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[2] This paper is Number 21 in the series "Studies on Plasma Proteins" from the Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross.

[3] Welch Fellow in Internal Medicine of the National Research Council, Member, Society of Fellows, Harvard University, during the first years of these investigations.

[4] It is unnecessary to give an extensive bibliography of the subject here. We cite, however, a few recent reviews by Eagle (1), Wöhlisch (2), Ferguson (3), Smith (4), Quick (5), and Taylor, Davidson, and Minot (6), which may serve as a guide to the present status of studies in this field.

which it moves with a characteristic mobility intermediate between that of the β- and the γ-globulins (7). In electrophoretic analysis of preparations containing fibrinogen, it is essential that the run be sufficiently long to effect adequate separation between fibrinogen and certain impurities whose mobility differs by as little as 0.5×10^{-5} cm.^{2} per volt per second at pH 7.7 and ionic strength 0.3.

Fibrinogen may also be determined by converting it to fibrin under suitable conditions, and determining the amount of protein nitrogen in the washed fibrin clot (8, 9). In normal citrated human plasma, quantitative clotting may be brought about simply by recalcification; in solutions of the plasma protein fractions, it is necessary to add a small amount of thrombin to secure quantitative clotting of the fibrinogen present.

The fibrinogen content of normal citrated human plasma was determined on pooled plasma samples, obtained from blood collected by the Boston Chapter of the American Red Cross and allotted to the Harvard pilot plant. The fibrinogen content of the 12 citrated samples, indicated in Table I, was determined as percentage of clottable protein. The average fibrinogen content from the 12 runs was 0.25 per cent, or slightly more than 4 per cent of the total protein. It is to be noted that, although 50 cc. of 4 per cent citrate were added to each bleeding, the volume of blood taken from individual donors varied and was not measured. Assuming an average bleeding of 500 cc., of which 55 per cent is assumed to be plasma, one obtains a value of 325 cc. citrated plasma per bleeding. The actual volume of plasma recovered per bleeding has been calculated, from a very extensive series of data, by Dr. H. B. Vickery. Recovery figures range from about 270 to 310 cc. per bleeding, so that a figure of 290 cc. is fairly representative of average recovery. This is 90 per cent of the total value of 325 cc., estimated above. On the basis of these assumptions, the fibrinogen content of uncitrated plasma would appear to be near 0.28 per cent. This figure agrees well with earlier careful measurements on normal plasma (10).

TABLE I

The fibrinogen content of citrated human plasma

Plasma Pool Number	Date	Percentage of fibrinogen
48	July 21, 1942	0.24
49	July 31, 1942	0.25
50	August 6, 1942	0.24
51	August 13, 1942	0.25
52	August 21, 1942	0.24
53	August 28, 1942	0.26
54	September 4, 1942	0.26
56	September 18, 1942	0.25
57	September 23, 1942	0.24
58	October 2, 1942	0.26
59	October 7, 1942	0.26
65	November 17, 1942	0.26
Average		0.25

By the methods of fractionation employed at the Harvard pilot plant, the major portion of the fibrinogen of plasma is concentrated in Fraction I. Moreover, of the total protein of Fraction I, approximately 60 per cent is fibrinogen, representing approximately a 14-fold increase in the proportion of this constituent in this fraction as compared with its concentration in whole plasma. Fraction I may be prepared as a dry sterile powder, which contains both proteins and salts, the latter being citrates or phosphates. In this form, the fibrinogen appears to remain stable for an indefinite period. On addition of distilled water, the powder readily redissolves, and the resulting solution is promptly clotted by the addition of thrombin. In the absence of added thrombin, the solution remains clear and does not clot for a period which is at least several hours and may be as long as 3 or 4 days. For most clinical applications, further purification is unnecessary; but by suitable further fractionation, preparations may be regularly obtained which contain 85 per cent or more of clottable protein. Determinations of fibrinogen content by prolonged electrophoresis and by the clotting method give very good agreement both for Fraction I and for more highly purified fibrinogen (Table II). In the instance of whole plasma, routine electrophoretic analysis yields a value for fibrinogen slightly higher than that obtained by the clotting method, owing to incomplete resolution of the fibrinogen peak from the slowest components in the β-globulins.

It is important also that Fraction I should contain as large a percentage as possible of the total fibrinogen in plasma. Under the best

TABLE II

Comparison of Tiselius analysis with clottable nitrogen

	Fibrinogen as determined by clottable nitrogen	Fibrinogen as determined by electrophoresis
	per cent	*per cent*
Preparation No. 27 III		
Fraction I	66.6	66
1st reprecipitation	76.1	75
2nd reprecipitation	84.4	84
Preparation No. 39		
5th reprecipitation	90.1	90

conditions so far worked out, the total yield in Fraction I approximates 75 per cent[5] (Table III).

The fibrinogen molecule is larger and far more elongated than any other plasma protein molecule. Its high asymmetry is shown by the fact that its solutions are highly viscous and show well-marked double refraction of flow.[6] These properties of fibrinogen, first recognized by other investigators, have been confirmed and extended by studies made in these laboratories. The molecular weight of fibrinogen is of the order of 500,000; the molecular length [7] is of the order of 900 Å. Thus the molecule may actually be regarded as a kind of miniature rod or fiber. Recent x-ray studies (12) confirm and amplify this conception, indicating that fibrinogen and fibrin both possess an underlying structural pattern analogous to that of the fibrous proteins, keratin and myosin. These studies indicate no fundamental difference in the detailed molecular pattern of fibrinogen and fibrin; the process of clotting appears rather to involve a linkage of the long fibrinogen molecules into far longer needle-like microcrystals. Elongated needles of fibrin have indeed been clearly seen directly by microscopic or ultramicroscopic observation in fibrin clots (13 to 15). Studies of the double refraction of oriented fibrin fibers (16) have also furnished clear evidence for the presence of elongated rod-shaped molecular structures, similar to those found in fibers such as those of muscle or tendon.

TABLE III

Fibrinogen concentration and percentage recovery of fibrinogen in whole plasma, found in Fraction I

Run no.	Fibrinogen	Recovered
	per cent	*per cent*
89	61.0	84
90	59.1	73
91	60.4	75
Average	60.2	77

The nature of the transformation of fibrinogen to fibrin, as induced by thrombin, is still obscure. Highly suggestive, however, are the recent experiments of Chargaff and Bendich (17) who have shown that such substances as ninhydrin and the sodium or potassium salts of 1,2-naphthoquinone-4-sulfonic acid, and 1,4-naphthoquinone-2-sulfonic acid produce a coagulation of fibrinogen which is strikingly similar to the natural fibrin clot. It is very suggestive that nearly all these substances can oxidize amino acids and peptides, containing free amino groups, with simultaneous decarboxylation. It is still too soon to judge whether a similar mechanism is involved in the action of thrombin on fibrinogen; the attempts of Chargaff and Bendich to detect a liberation of CO_2 during this process led to inconclusive results. We may hope, however, for a deeper understanding of the chemistry of the process in the near future.

Some of the diverse uses of the fibrin clot, and the modified forms in which it may be obtained, are discussed in Papers XVI to XXI of this series (18 to 23).

PROTHROMBIN

The first prothrombin preparations of high potency were obtained by Mellanby (24), the initial step in his process being dilution of the plasma, and precipitation of prothrombin by adjustment of pH to 5.3. His work indicated clearly that prothrombin is a euglobulin with a point of minimum solubility close to this pH. Prothrombin is very readily adsorbed by many reagents, such as the insoluble hydroxides and silicates of magnesium and aluminum. Adsorption and elution, together with other procedures,

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[5] We may also call the reader's attention to a recent simple method for preparing human fibrinogen of high tensile strength, described by Neurath, Dees, and Fox (11).

[6] See Wöhlisch (2), pp. 277–293; and for the general significance of these properties, see Paper I of this series (7).

[7] From unpublished double refraction of flow measurements by I. H. Scheinberg in this laboratory.

have been skillfully employed by Seegers (25) in obtaining the most active prothrombin preparations yet achieved. The material obtained by Seegers was a protein containing about 4 per cent carbohydrate. Its solubility behavior was similar to that indicated by Mellanby's work, and it was found to be very unstable in solutions acid to pH 4.8, or alkaline to 9 or 10. Like thrombin, it was nondialyzable.

In the process of fractionation employed in these studies (7), prothrombin was found to be concentrated in the proteins of Fraction III-2. Its activity in this fraction, expressed in prothrombin units [8] per milligram of protein, is generally from 12 to 15 times as great as in the original plasma, and in favorable cases is considerably higher than this.

The possibility of injecting concentrated prothrombin solutions into patients suffering from prothrombin deficiency has frequently been suggested as a therapeutic measure, for while certain of such deficiencies are frequently and readily treated with vitamin K, cases occur in which such therapy is without effect, notably in the presence of reduced liver function. The risk of intravascular clotting from prothrombin injections must, of course, be considered a very serious one. The preparations of prothrombin obtained by us are as yet to be considered unsafe for intravenous use. They are unstable from two standpoints: first, the preparation rapidly loses activity unless the prothrombin is converted to the far more stable thrombin; secondly, spontaneous formation of small quantities of thrombin occurs without the addition of any converting agent. Work aimed at the elimination of these drawbacks (which are shared by practically all prothrombin preparations hitherto reported) is now in progress.

[8] We have followed the definition of Seegers, Smith, Warner, and Brinkhous (26) who state "One unit of prothrombin is that amount which, when completely converted into thrombin will clot 1 cc. of fibrinogen solution in 15 seconds." The concentration of fibrinogen has little influence on the clotting time provided it lies in the range 0.3 to 1.0 per cent. However, the type of fibrinogen employed, and the method of its preparation, may considerably influence the results obtained. Also the criteria of clot formation, used in evaluating clotting times, have been chosen differently by different workers. Therefore, the value of the prothrombin unit, by the above definition, is probably not the same in different laboratories; although highly consistent results can be attained by the workers in any one laboratory, using a carefully standardized technic.

The unit of thrombin is determined in exactly the same way as that of prothrombin, except that the solution to be tested is added directly to the fibrinogen solution, without any attempt to convert the prothrombin present at the time of addition.

THROMBIN

A variety of uses has been found (18, 21, 22) for the prothrombin present in Fraction III-2 when converted into thrombin. Bovine thrombin of very high potency has been prepared by Seegers (25 to 27) and by Milstone (28). The methods of purification, although in many ways radically different, yield products of comparable activity. The thrombin of Seegers appears to be somewhat the more potent of the two. The chemical properties of thrombin are in many ways similar to those of prothrombin. Its pH of minimum solubility, however, is reported to be more acid (25); and it is distinctly more soluble than prothrombin at almost all pH values and salt concentrations. Like prothrombin, however, it is an euglobulin—that is a protein which at or near its isoelectric point is only slightly soluble in water, but is much more soluble in dilute salt solution. The work of Milstone (28) has shown that thrombin is not so readily salted out as prothrombin. Thrombin is soluble in 0.45 saturated ammonium sulfate solution, whereas crude prothrombin is not. Thrombin is also considerably more stable than prothrombin, particularly in acid solutions.

A much less highly purified, but extremely simple and clinically effective preparation, was obtained by Parfentjev (29) who fractionated rabbit plasma with ammonium sulfate, and obtained a fraction high in thrombic activity, which retained its activity in solution over a long period of time. Since the original plasma, before fractionation, contained little or no thrombin, it is probable that the fractionation procedure effected the conversion of prothrombin to thrombin, in addition to separating the resulting thrombin in one of the fractions obtained. The mechanism of the effect remains unexplained, but Parfentjev's findings were confirmed and extended by Taylor, Lozner, and Adams (30); and Lozner, MacDonald, Finland, and Taylor

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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

XVI. FIBRIN CLOTS, FIBRIN FILMS, AND FIBRINOGEN PLASTICS[1,2]

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(Received for publication February 17, 1944)

The first papers in this series (1 to 5) have described the properties of the constituents of plasma in solution. The physical chemical properties of sedimentation, electrophoretic mobility, osmotic pressure, viscosity, and double refraction of flow reflect the behavior of the protein molecules moving as individuals in the dissolved state. The physiological properties which are of importance in medicine, such as restoration of blood volume, immunization, and agglutinin reactions, are presumably related to individual molecular behavior.

Highly specific properties of certain molecules, such as fibrinogen, lead to their association to form solid structures. This introduces a new set of physical chemical properties, and a new set of physiological properties which apply to surgery rather than to medicine. The preceding paper has dealt with the physical chemistry of the normal clotting process (6). Now, it has been found that, when the human plasma proteins which are involved in the normal process are allowed to react *in vitro*, under controlled conditions, clots may be obtained with a wide variety of physical characteristics. We shall describe these clots, as well as two kinds of derived products—the fibrin films and fibrinogen plastics—which we have developed from the same proteins.

The clots, films, and plastics are structural materials whose value depends upon a combination of histological behavior following implantation in tissue with certain mechanical properties. The histological and clinical sequences are the subject of other communications in this series (7 to 11). The mechanical properties, which can be controlled to provide a wide variety of structures, are treated here.

The clots, films, and plastics are derived from Fraction I of human plasma (1), which consists largely of fibrinogen. In the *clots* and *films*, the fibrinogen has been converted to fibrin, by the action of thrombin (Fraction III-2). In the *plastics*, the fibrinogen has not been clotted, nor ordinarily separated from accompanying globulins, but the entire fraction has been subjected to an irreversible molding treatment.

FIBRIN CLOTS

The fibrin clots are comparatively tenuous structures, and only a small proportion of their volume—generally 2 per cent or less—is occupied by the fibrin which is responsible for their solidity. They are far less stable than the films and plastics, and are ordinarily prepared immediately before use, by mixing appropriate sterile solutions of human fibrinogen and thrombin. In clinical use, they may be formed *in situ*, the solution mixture being allowed to clot in a cavity or on a surface according to the nature of the treatment (8, 12).

The physical properties of the clots may, according to conditions of preparation, be graded continuously between two extremes, which for convenience are designated Type A and Type B. The former are transparent, gelatinous, and friable; have low tensile strength and maximum elongation (*i.e.*, cannot be stretched very far); adhere well to surfaces upon which they are formed; and do not readily synerize (*i.e.*, lose

[1] This work was originally supported by grants from the Rockefeller Foundation and from funds of Harvard University. Since March, 1942, it has been carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[2] This paper is Number 22 in the series "Studies on Plasma Proteins" from the Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross.

water with a contraction in volume). The latter (Type B) are opaque, doughy, and non-friable; have a high elongation, and plastic flow; do not adhere well to surfaces upon which they are formed; and synerize very readily.

The clotting times involved in the formation of the clots may be varied. Their order of magnitude may range from a few seconds to 15 minutes, for those of Type A; and from a minute to an hour, for those of Type B.

FIBRIN FILMS

The term fibrin film is applied both to a single lamina of plasticized fibrin and to modified sheets, consisting of fibrin backed by other layers (bandage, waterproofing, etc.). The plain fibrin films form the basic material for the other types. They can provide a variety of mechanical properties, ranging from a soft and resilient to a tough and rubbery structure. Products with different properties may be prepared to meet the mechanical and chemical specifications for different clinical needs.

Dimensions. Plain fibrin films have thus far been prepared in sheets, seamless tubes, and fibers. The sheet thickness is usually specified in terms of milligrams of fibrin per square centimeter, which may range from 1 to 50. These figures, multiplied by 0.03, give the approximate thicknesses in millimeters (Figure 1). The tubes have been prepared with different diameters (*e.g.*, 2 to 20 mm.) and wall thicknesses (*e.g.*, 0.1 to 1.5 mm.). Several sizes are shown in Figure 2. The fibers have been prepared with diameters ranging from 0.2 to 1.0 mm. (Figure 3).

Composition. The films are composed of protein plus plasticizer. A "plasticizer," in technical terminology, is a substance of low molecular weight (usually a liquid) which is incorporated to make a plastic material soft and flexible rather than hard and brittle. In the fibrin films, the plasticizer may be simply water. If, for storage and distribution, other liquids are employed, due consideration has been given to their lack of toxicity, local and general. As used in surgery, the films are frequently immersed for some time in water or saline; this procedure

FIG. 1. FIBRIN FILMS, IN SHEET FORM

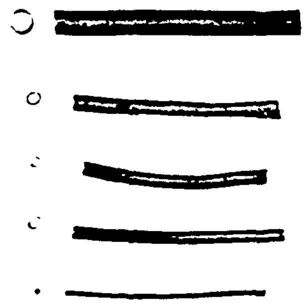

FIG. 2. FIBRIN FILMS, IN SEAMLESS TUBING FORM

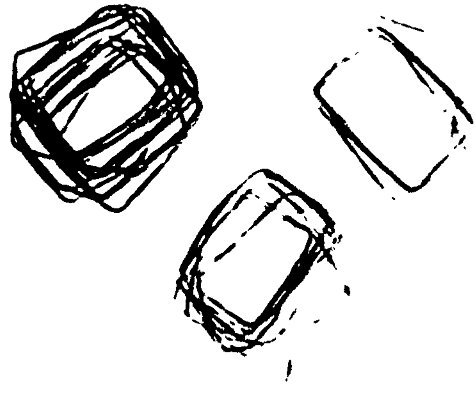

FIG. 3. FIBRIN FILMS, IN FIBER FORM

removes part or all of any other plasticizer and replaces it by water. The protein moiety is ordinarily at least 90 per cent fibrin, and the occluded human globulin may, as a first approximation, be neglected.

The proportion of fibrin may vary from 15 to 100 per cent by weight, the remainder being plasticizer. The mechanical properties depend to a very great extent upon these proportions, that is, upon the extent to which the fibrin units are diluted and forced apart by the liquid plasticizer.

Mechanical properties. The mechanical properties of the fibrin films can best be described by stress-strain curves, in which the stress (or load per *original unstretched* cross-section area) is plotted against percentage elongation (the increase in length expressed as percentage of the original length). Similar curves are commonly used to describe the behavior of rubber and synthetic plastics, as well as natural and synthetic fibers.

The shape of the stress-strain curve is primarily influenced by the proportion of fibrin in the film.

When the amount of fibrin is *small*, and the plasticizer occupies most of the volume, the stress increases linearly until an elongation of a little over 100 per cent is reached, and then goes up much more rapidly (Figure 4). Thus the film has an easy rubbery stretch to 100 per cent, and thereafter "firms up" so that further stretch is more difficult. This type of curve we have called *linear.* Sometimes films break before the firming-up stage is reached (Figure 4a) but this is attributed to flaws rather than to any essential difference in structure.

When the amount of fibrin is *large*, the stress-strain curve rises steeply at first, then at about 30 per cent elongation flattens off, and finally rises steeply at the end (Figure 5). Thus, the film, which may be qualitatively described as "tough" rather than "rubbery," requires a strong pull at the beginning of stretch, then deforms more easily as the stretching continues, and eventually firms up at 100 to 150 per cent elongation. This type of curve is described as *S-shaped.*

The difference between linear and S-shaped stress-strain curves may be interpreted in terms of the attractive forces between the units of the fibrin structure (whether these units are molecules, micelles, or other aggregates, or crystallites, remains to be determined). When the units are kept apart, initial stiffness is avoided; when the units are closer together, the attraction between them requires a high initial stress to start deformation.

It is of interest to compare the linear and S-shaped curves of Figures 4 and 5 with the stress-strain curves of certain natural biological

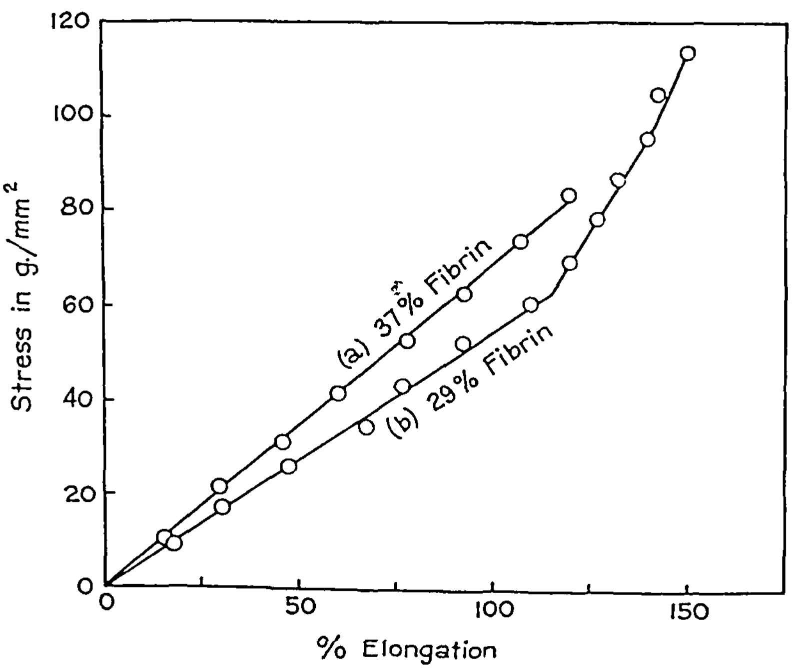

FIG. 4. STRESS-STRAIN CURVES OF FIBRIN FILM (LINEAR TYPE)

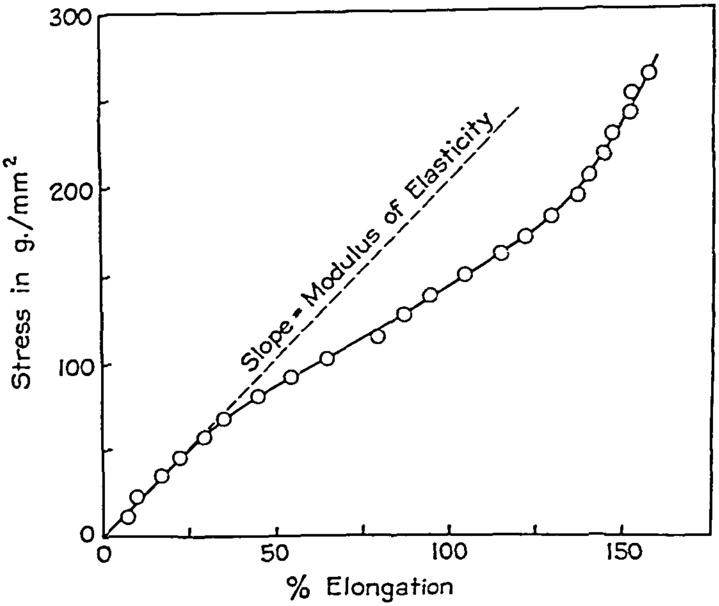

FIG. 5. STRESS-STRAIN CURVE OF FIBRIN FILM (S-SHAPED TYPE)

structures. The linear curve (Figure 4b) is very like that of the *ligamentum nuchae* (13) (Figure 6), whereas the S-shaped curve (Figure 5) is very like that of a wool fiber (14) (Figure 7) except for a difference in scale. The *ligamentum nuchae*, of course, normally contains a large amount of plasticizer (*i.e.*, tissue fluids); whereas wool has a rather low content of plasticizer (*i.e.*, moisture).

The mechanical behavior of either of these natural structures can thus be imitated by the fibrin film, and it may be possible to prepare types of film to simulate other anatomical structures if the mechanical specifications are provided.

From the stress-strain curve, three characteristic constants of a film can be evaluated: the modulus of elasticity, which is the initial slope, the tensile strength, or stress at break, and the maximum elongation at break. The last two are less reproducible than the modulus, since the point of break may depend upon the presence of a flaw rather than the yielding of a homogeneous protein structure.

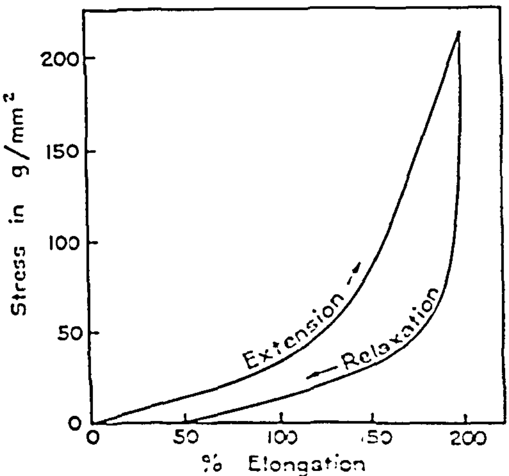

FIG. 6. STRESS-STRAIN CURVE OF *LIGAMENTUM NUCHAE* (BOVINE)

After Wöhlisch, du Mesnil de Rochemont, and Gerschler (13).

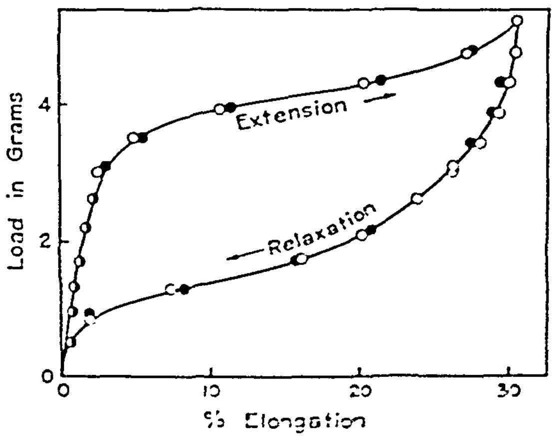

FIG. 7. STRESS-STRAIN CURVE OF WOOL FIBER

After Harris, Mizell, and Fourt (14).

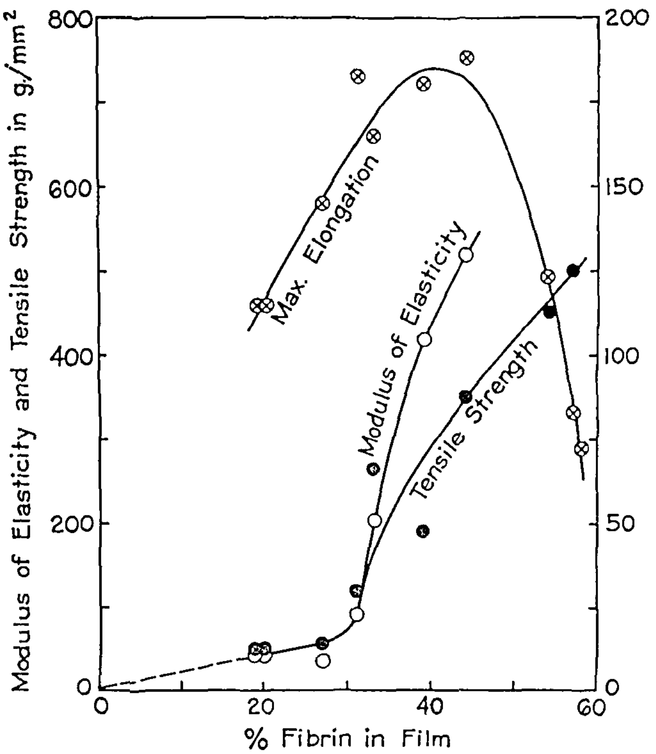

FIG. 8. MODULUS OF ELASTICITY, TENSILE STRENGTH, AND MAXIMUM ELONGATION OF FIBRIN FILM, PLOTTED AGAINST PERCENTAGE FIBRIN IN THE FILM

The three characteristic constants depend enormously upon the proportion of fibrin in the film, as shown in Figure 8. The modulus and the tensile strength increase slowly at first, and then rise very rapidly when a certain critical concentration of fibrin is exceeded. The maximum elongation goes through a maximum as the proportion of fibrin in the film is increased. This phenomenon is easily explained. When the fibrin concentration is small, the film is soft, and the coherence of the fibrin units is so weak that they pull apart before a high elongation is reached. At an intermediate concentration, the coherence is strong enough to allow a high elongation while the units still hang together, but not strong enough to interfere with the considerable internal rearrangement of molecules which must occur when a body is stretched to three times its original length. At a still higher fibrin concentration, the attractions are so strong that the internal rearrangements cannot take place; the film supports a high stress but snaps before it has stretched very far.

Chemical properties. The chemical properties of the fibrin films may be altered by various treatments. For example, the untreated film is readily digested by proteolytic enzymes, including trypsin, pepsin, the natural lysin which is concentrated in some of the fractions of blood plasma (1, 6), and the lytic enzyme of hemolytic streptococcus.[3] A small amount of the natural human plasma lysin, occurring largely as an impurity in the thrombin used to form the fibrin, is often retained in the film, so that the latter may lyse spontaneously when plasticized with water alone. Certain of the other plasticizers that have been employed inhibit the natural lysin and are therefore used in storage.

After certain treatments, the film becomes increasingly resistant toward proteolytic enzymes, and also toward absorption in living tissue.

Modified fibrin films. Besides the plain Type P fibrin film, modifications have been prepared as follows:

Type F film consists of plasticized fibrin with a backing of elastic bandage or elastic knitted cotton material for use in bandaging (Figure 9).

Type W film consists of plasticized fibrin with an elastic waterproof covering, made of non-toxic synthetic plastic, selected to have the same stress-strain characteristics, as far as possible, as the fibrin itself.

Type WF film consists of plasticized fibrin with a backing of elastic bandage or elastic cotton material, to the other side of which is applied an elastic waterproof coating.

The possible value of these fabric-backed and waterproof films is discussed in another paper of this series (9).

Sterilization. Fibrin films of certain of the types described above have been prepared asep-

FIG. 9. TYPE F FIBRIN FILM (BACKED BY ELASTIC KNITTED COTTON)

[3] Prepared by Dr. E. A. Bering, Jr.

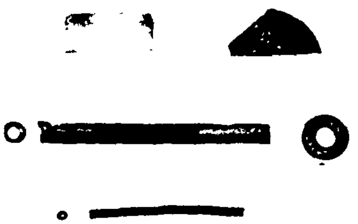

FIG. 10. FIBRINOGEN PLASTICS

tically. For large scale production, however, procedures for sterilization of the films after manufacture are desirable. Several methods have been attempted, some of which, successful on an experimental scale, have yielded the products used clinically (11). Investigations of the most satisfactory methods of sterilizing each type of film are still in progress.

FIBRINOGEN PLASTICS

The fibrinogen plastics are thermosetting, *i.e.*, they are set in molds under the action of heat, but cannot be softened or remolded subsequently.

Composition. The fibrinogen plastics, like the films, are composed of protein plus plasticizer. The proportion of protein may range from 25 to 75 per cent. The protein moiety is ordinarily Fraction I. Fibrinogen purified from Fraction I may also be employed. When plastics are used in surgery, the original plasticizer may be to a large extent replaced by water if the material is immersed for some time in water or saline before introduction into the body. After introduction into the body, the plasticizer will eventually be replaced by tissue fluids in any case.

The high permeability of fibrinogen plastics to water may be contrasted with the behavior of rubber and many synthetic plastics. Whereas the latter, generally speaking, are impermeable to water but permeable to oils and hydrocarbons, the fibrinogen plastics are permeable to water but impermeable to oils and hydrocarbons.

Optical properties. The plastics are translucent to light. Unlike the films, which are almost colorless, the plastics range in color from straw to dark brown. Like the films, they become doubly refracting under stress.

Mechanical properties. As in the case of films, the mechanical properties of the plastics depend largely upon the relative proportions of protein and plasticizer. As an approximate description, those containing 25 to 50 per cent protein are rubbery, those containing 50 to 75 per cent protein are leathery, and those containing more than 75 per cent protein are horny in character.

The stress-strain curves are similar to those of the films, except that the maximum elongation is much less, rarely exceeding 50 per cent.

SUMMARY

Fibrin films and fibrinogen plastics are structural materials derived from the proteins of human plasma involved in the natural coagulation process. They may be prepared in a wide variety of shapes, sizes, and mechanical properties. In the latter characteristics, which are dependent largely upon the proportion of protein present in the solid mass, the films and plastics may simulate different natural anatomical structures. It is possible to modify their susceptibility to attack by proteolytic enzymes, as well as their rates of absorption in living tissues.

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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

XVII. A NOTE ON THE ABSORPTION RATES OF FIBRIN FILMS IN TISSUE[1,2]

By PETER R. MORRISON and MARCUS SINGER

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(Received for publication February 17, 1944)

The most effective surgical use of fibrin film (1) or foam (2) demands some knowledge of their rates of absorption in the body.

Fundamental observations on the histological sequences involved in the course of absorption of fibrinogen plastics (1) implanted in tissue have been made by Bailey, Ford, and Hawn (3).

The present study was undertaken to measure the rates of absorption of various types of fibrin films, so that it might be possible to adjust the persistence of film in the body to meet specific requirements.

MATERIALS AND METHODS

The fibrin film used in these experiments was that described in the preceding paper (1), prepared from human fibrinogen (4, 5). It was subjected to varying degrees of modifying treatment before implantation. Squares of film (1 cm. × 1 cm.) were then implanted subcutaneously and intermuscularly in rabbits and held in place by silk sutures. In order to determine the initial fibrin content of the implanted specimens, similar pieces of film were soaked in distilled water to free them of water-soluble substances, dried at 110° C., and weighed to 0.3 mgm. This method was reproducible within 5 per cent. Three different weights of film were employed, with 4, 9, and 11 mgm. fibrin per sq.cm. The final fibrin content of the films was determined after different periods of implantation by submitting the remains of the films to the soaking, drying, and weighing procedure described above. In cases where encapsulation occurred, the weight of the capsule was measured separately after soaking and drying. Whereas the film may be easily recovered after brief periods of implantation, it becomes increasingly difficult to identify as absorption proceeds, owing to fragmentation and possible invasion of fibrous tissue. Hence, the results for the long periods of implantation must be considered as less reliable than those obtained for short periods.

RESULTS

The results demonstrated that a considerable range of persistence times may be obtained, depending on the extent of modifying treatment of the films. Untreated film was absorbed most rapidly; the rate of absorption decreased with an increasing degree of treatment.

Untreated films. Complete absorption of all the untreated films (4, 9, and 11 mgm. fibrin per sq.cm.) occurred in less than 9 days. Even at the earliest time of examination (5 days), the film was very weak and could not be removed in a single piece. The rate of absorption of each film was roughly constant over the entire period. It varied from 0.85 mgm. per sq.cm. per day for the 4 mgm. per sq.cm. film to 1.40 mgm. per sq.cm. per day for the 11 mgm. per sq.cm. film,

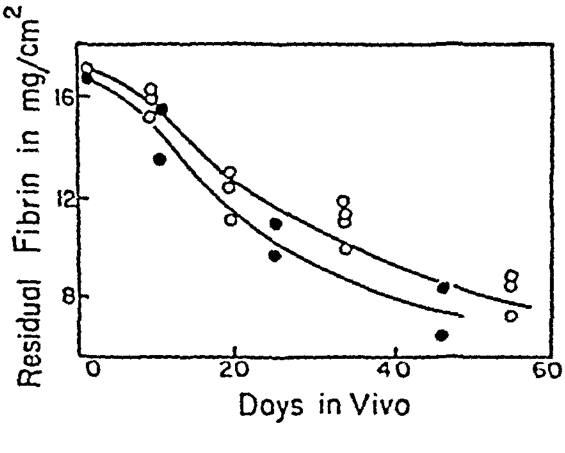

FIG. 1. COURSE OF ABSORPTION OF TREATED FILMS: RESIDUAL FIBRIN PLOTTED AGAINST TIME OF IMPLANTATION IN DAYS

Initial weight 17 mgm. per sq. cm. per day.

[1] This work has been carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[2] This paper is Number 23 in the series "Studies on Plasma Proteins" from the Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross.

the rate thus being not quite proportional to the original thickness.

Treated films. The rate of absorption of the films with the greatest modifying treatment was much slower than the above and fell off progressively with time (Figure 1). These films disappeared initially at the rate of 0.25 to 0.35 mgm. per day; by 40 days, however, the rate had fallen to 0.12 to 0.14 mgm. per day. Total

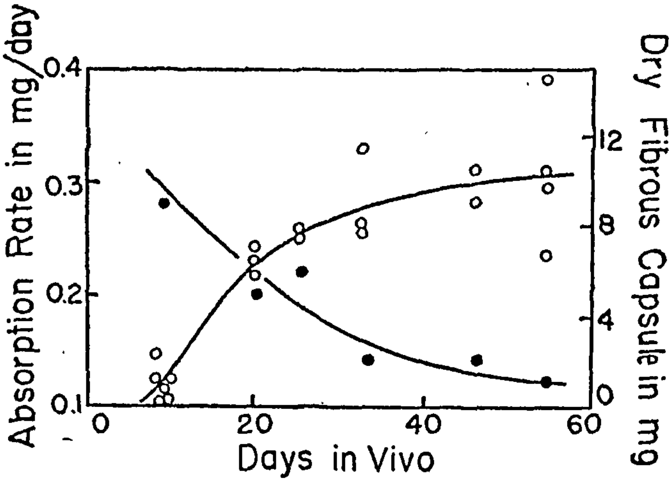

FIG. 2. ABSORPTION OF TREATED FILMS AND DEVELOPMENT OF CAPSULE

Rate of absorption of films in mgm. per sq. cm. per day—●. Dry weight of capsule in mgm.—○.

absorption of the film was not complete even in the one animal allowed to live for 81 days after implantation. The fall in absorption rate was associated with the development of a capsule, which was easily separable from the surrounding

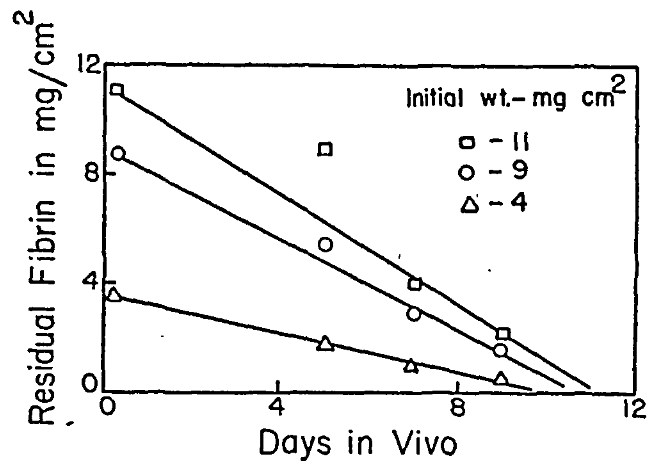

FIG. 3. COURSE OF ABSORPTION OF FILMS WITH SLIGHT TREATMENT: RESIDUAL FIBRIN PLOTTED AGAINST TIME OF IMPLANTATION

Initial weights of 4 (△), 9 (○), and 11 (□) mgm. per sq. cm.

loose connective tissue and completely enclosed the film (Figure 2). The latter, in turn, lay free inside the capsule, and, unlike the untreated film, could usually be removed in a single piece, although its tensile strength had dropped considerably. It is possible that the decrease in rate of absorption was due merely to the formation of the capsule and not to any difference in the absorption process.

Intermediate degrees of modifying treatment produced films with intermediate absorption rates. The absorption curves for slightly treated films were similar to those of untreated film (*Figure 3*).

COMMENT

The range of absorption rates (from 0.2 to 1.4 mgm. per sq.cm. per day) shown by the various types of film, in combination with different weights of film available, offer a wide latitude in choosing a film for any specific purpose.

The fact that the absorption rate, when expressed in mgm. per sq.cm. per day, is roughly proportional to the initial film weight for untreated films and also for those of slight degrees of treatment, means that the absorption rates, expressed as per cent of original weight, and the total persistence times, are essentially independent of film weight. This suggests that the absorptive process involves the entire film simultaneously and is in all probability not localized at the surface.

Although the extent to which the results of animal experiments with a heterologous protein can be applied to man is never certain, histological and gross observations on clinical material (6) suggest that, in general, absorption rates and types of reaction to the films are qualitatively similar, though quantitative differences may well obtain between different species, and between different tissues of a given species.

SUMMARY

The rates of absorption of fibrin films in tissue have been measured.

Persistence times, ranging from less than 5 days for untreated fibrin film to more than 80 days for fully treated films, have been observed. For films of extended persistence time, an encap-

sulation occurs between 10 and 30 days after implantation. The choice of material for surgical use should be guided by the persistence time desired for each specific application.

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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

XVIII. FIBRINOGEN COAGULUM AS AN AID IN THE OPERATIVE REMOVAL OF RENAL CALCULI [1]

By JOHN E. DEES

(From the Urologic Division, Department of Surgery, the Duke University School of Medicine and Duke Hospital, Durham, North Carolina)

(Received for publication February 17, 1944)

In spite of the fact that the surgeon operating to remove stones from the kidney has at his command the invaluable aid of either the fluoroscope or the x-ray film used at the operating table as a means of determining the number of stones present and their location in the ramifications of the renal pelvis, he still may find their extraction difficult and accompanied by an undesirable degree of injury to the renal parenchyma. The use of grasping forceps is difficult at times or even dangerous because of the chance of crushing the stone and leaving unsuspected minute fragments to serve as nuclei for further growth. Irrigation of the pelvis with sterile saline solution followed by negative pressure suction through a tube inserted into the various compartments may also fail to remove all concretions.

Still another aid in the removal of fugitive or inaccessible calculi has been devised by means of a coagulum of fibrinogen, caused to form in the pelvis, which on removal will bring away the calculus incorporated within itself (Figure 1).

The preparation and properties of the coagulum, as well as the first five clinical cases, have been described elsewhere (1 to 3). Subsequently, fibrinogen separated in Fraction I in course of large scale plasma fractionation (4, 5) has been employed to prepare coagula with mechanical properties (6) adapted to the procedure. Fibrinogen dissolved in a buffer solution is injected into the renal pelvis in an amount sufficient to fill it fully while at the same time a small amount of thrombin ("clotting globulin") is simultaneously injected. The resultant clot is complete after the expiration of 4 to 5 minutes and is then withdrawn. The coagulum has a tensile strength from 10 to 20 times greater than that of a blood clot, and is unaffected by the presence of small amounts of urine or of blood. After from 6 to 24 hours, however, at body temperature, the clot becomes disintegrated by the action of urine. Postoperative observation of patients, as well as that of the experimental animal, shows that the coagulum has no irritative effect whatever on the pelvic epithelium.

The use of such a coagulum has the following advantages: (*1*) All free stones, regardless of size, number, or position within the renal pelvis, should be removed. (*2*) Fragmentation of calculi during removal is avoided. (*3*) Trauma to the kidney is reduced to a minimum. (*4*) Complete surgical mobilization of the kidney may be unnecessary, as exposure of the renal pelvis alone provides adequate exposure for the procedure. The coagulum cannot be expected to remove a calyceal calculus of larger diameter than that of the infundibulum through which it must pass, nor a calculus imbedded in or adherent to the wall of the pelvis.

In clinical cases where infection was absent, or only moderate, the coagulum has been almost uniformly successful in incorporating all free stones. In instances where severe infection was present and the urine had become mucoid, slimy, and thick, the clot has failed to incorporate a certain number of free stones. We have felt that these failures were due to a difference in surface tension of fibrinogen solution and mucoid urine which prevented complete dissemination of the injected fluid throughout the pelvis and around

[1] Certain of the products of plasma fractionation employed in this work were developed from blood, collected by the American Red Cross, by the Department of Physical Chemistry, Harvard Medical School, Boston, Massachusetts, under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

Calculi Removed With Coagulum

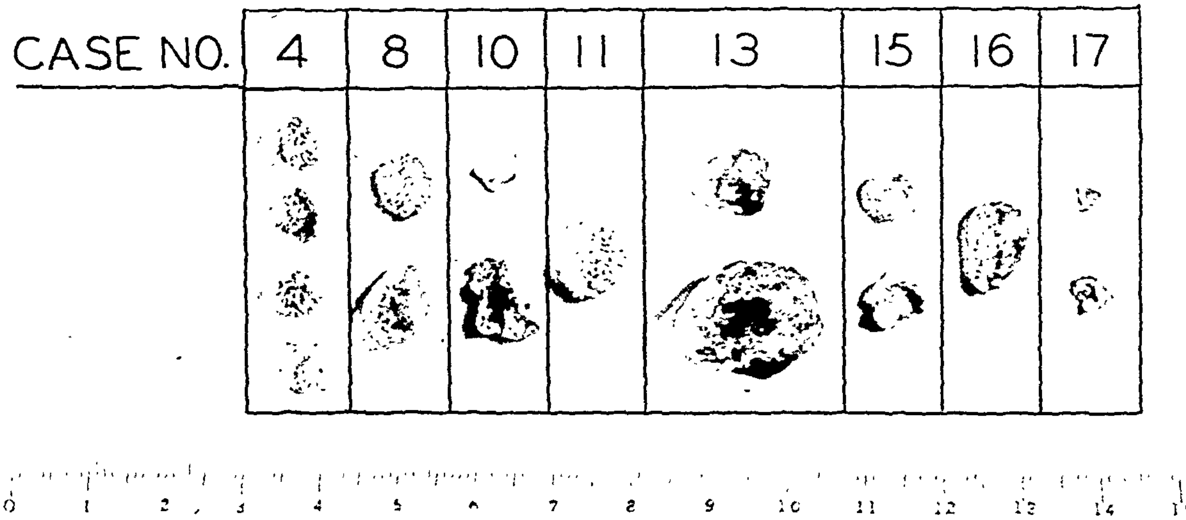

Fig. 1. Photograph of Renal Stones Removed by Means of Coagulum

each calculus. To overcome this difficulty, a number of wetting agents and detergents have been investigated, the most satisfactory so far examined being Aerosol O.T.[2] Toxicity experiments, to be reported elsewhere, indicate that the use of this substance in the kidney pelvis, as herein described, is without ill effect. *In vitro* experiments and its use in 5 clinical cases strongly suggest that preliminary irrigation of the kidney pelvis with 0.1 per cent Aerosol solution greatly improves the distribution of fibrinogen solution throughout the pelvis.

The steps in the operative procedure in use at the present time are as follows: (*1*) After surgical exposure of the kidney pelvis and upper ureter, the lumen of the ureteropelvic junction is gently occluded with a suitable instrument or tape to prevent escape of the coagulable material down the ureter. (*2*) A small incision, 4 mm. in length, is made in the renal pelvis. (*3*) A No. 12 French rubber urethral catheter with two eyes is introduced through the small incision until the second eye lies just within the renal pelvis. If it does not fit the opening snugly, it may be made to do so by grasping one end of the incision with a mucosa clamp. Urine is then aspirated from the pelvis. (*4*) The kidney pelvis is next filled with normal saline solution and completely aspirated by means of a graduated syringe. The amount of normal saline necessary to distend the pelvis fully may be taken as the pelvic capacity. The catheter should be so adjusted that it irrigates perfectly. (*5*) The pelvis is next lavaged with a solution of 0.1 per cent Aerosol, in an amount not exceeding two-thirds of its predetermined capacity. (*6*) Lavage is next carried out with a solution of fibrinogen to insure complete distribution of this material throughout the pelvis and around the calculi. It is then withdrawn by aspiration. (*7*) Fibrinogen solution in an amount equal to approximately 90 per cent of the predetermined capacity of the renal pelvis is next injected through the urethral catheter into the pelvis. Simultaneously, by means of a 2 cc. syringe and needle, an assistant injects one-tenth that amount of 2 per cent clotting globulin[3] through the wall of the urethral catheter into its lumen, so that the two materials are intimately mixed as they enter the renal pelvis. The fibrinogen solution should be approximately 37° C. at the time of injection. (*8*) Four minutes are allowed to elapse. (*9*) After removing the urethral catheter, the usual pyelotomy incision is made by

[2] The di-octyl ester of sodium sulfosuccinic acid, a product of the American Cyanide and Chemical Corporation, New York City.

[3] I am indebted to Lederle Laboratories, Inc., for generous quantities of clotting globulin.

extending the initial small incision as desired. (*10*) The coagulum partially extrudes through the pyelotomy opening, and may be grasped with ring forceps and slowly but firmly withdrawn. It should remove, enmeshed within itself, all free stones from the renal pelvis. (*11*) The pyelotomy incison is usually closed only partially to insure against ureteral obstruction during the first postoperative day by any portion of the clot which might remain in the pelvis. Several cases have been closed completely, however, without ill effect.

The clinical results in 21 patients for whom the coagulum was used are as follows: In 13 patients, all stones were removed; and in 3 of these patients, additional tiny stones, not demonstrated in the pre-operative x-ray film, also came away. In 6 patients, some but not all of the stones were removed. In 2 instances, the coagulum failed to remove free stones.

An illustrative case history is that of a 27-year-old man who had an irregular calculus, measuring 12 × 9 × 6 mm., partially obstructing the right lumbar ureter which had been producing intermittent symptoms for 8 months. In addition, a very faint opacity suggestive of calculus was visible by x-ray in the region of the lower calyx of the kidney above (Figure 2A). The renal pelvis was moderately hydronephrotic. At operation, the ureteral calculus was removed through a ureterotomy incision made directly over the stone. Then, by exposing the extrarenal portion of the pelvis, coagulum pyelolithotomy was carried out, the capacity of the pelvis being 25 cc. On removing the coagulum, 2 irregular small calculi were found imbedded within it (Figure 2B). One calculus measured 4 × 4 × 2 mm.; the second stone measured 3 × 2 × 1 mm.

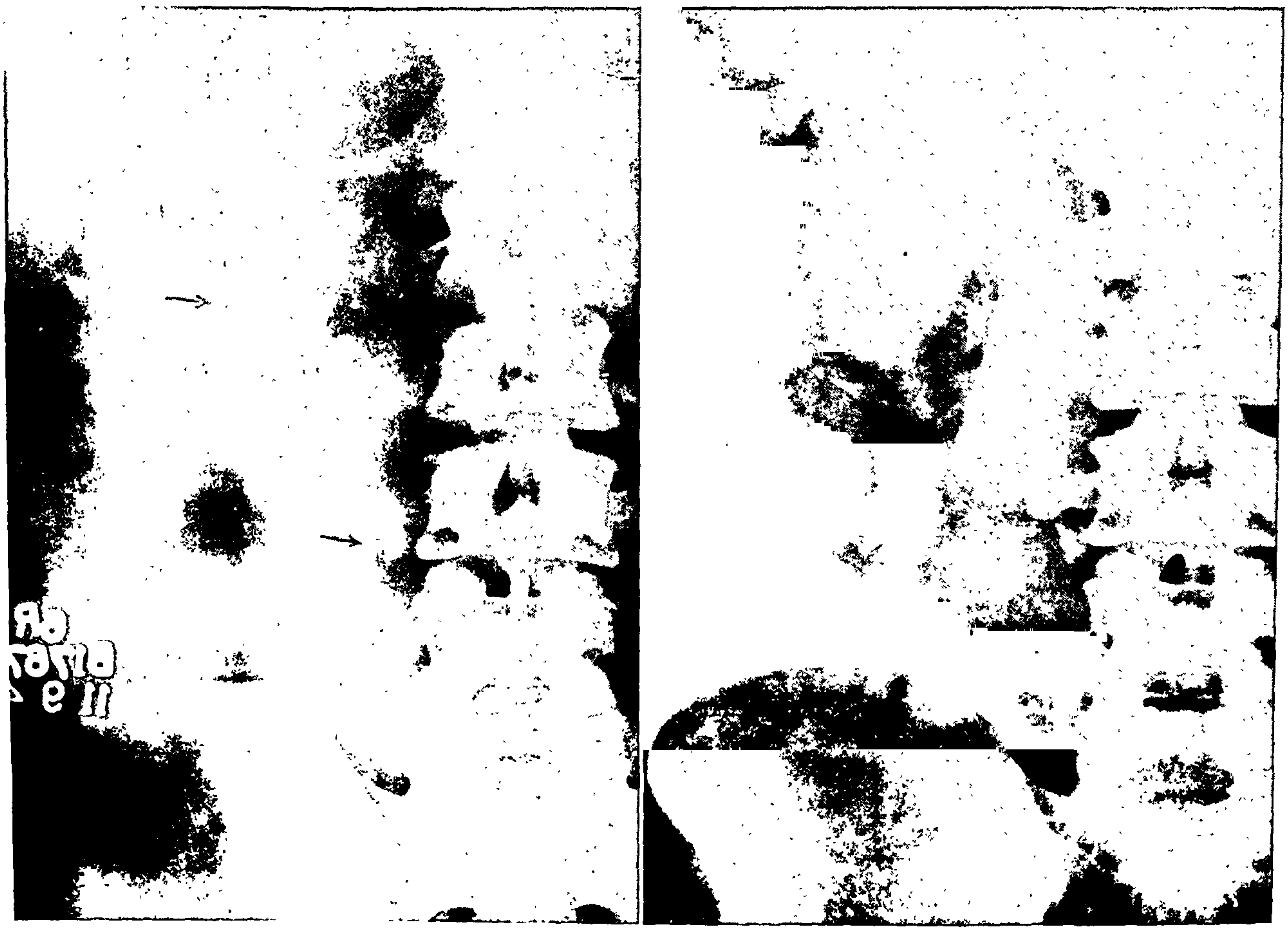

Fig. 2A. Plain X-Ray and Intravenous Urogram Showing Lumbar Ureteral Calculus, Hydronephrosis, and Faint Opacity, Suggestive of Calculus in Region of Lower Renal Calyx

Fig. 2B. Photograph and X-Ray of Coagulum Showing Removal of 2 Tiny Stones from Right Kidney Pelvis (Ureteral Calculus Removed Separately)

SUMMARY

A new aid in the removal of small free stones from the renal pelvis at open operation is described. By the simultaneous injection of solutions of fibrinogen and thrombin, a strong coagulum which completely fills the pelvis and enmeshes all free stones is produced. On withdrawing this coagulum through the usual pyelotomy incision, all free stones should be removed. Fragmentation of calculi and trauma to the kidney are thus avoided. This operative procedure has been carried out in 21 patients without demonstrable ill effect. The operative results herein reported, although still imperfect, are improving as additional experience with the method is acquired. It is hoped that this procedure will be of distinct aid in the problem of the surgical removal of renal calculi.

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XIX. A NOTE ON THE USE OF FIBRINOGEN AND THROMBIN IN THE SURFACE TREATMENT OF BURNS [1,2,3]

By CLINTON v. Z. HAWN, EDGAR A. BERING, JR.,[4] ORVILLE T. BAILEY, AND S. HOWARD ARMSTRONG, JR.[5]

(From the Departments of Pathology and Physical Chemistry, Harvard Medical School, Boston)

(Received for publication February 17, 1944)

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1. It should lack the deleterious effect on healing possessed by the conventional escharotics (1 to 3).
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At this time it became apparent that large quantities of purified human fibrinogen and thrombin were becoming available through the fractionation of human plasma to prepare albumin for the armed forces (4, 5). Studies, therefore, were undertaken to develop from these proteins, which constitute important components in the natural mechanism for the protection of wounds, a dressing which would meet these specifications.

THE HEALING PROCESS UNDER FIBRINOGEN-THROMBIN FILMS

The initial phases of the work which were carried on both in animals and men were aimed at determining whether fibrinogen-thrombin mixtures, together with other substances as plasticizers, met the first of the specifications, namely, that they have no deleterious effect on the healing process.

Animal experiments. The nature of the skin surface of common laboratory animals is such that blistering and loss of serum do not follow a burn with the exception of such regions as between the toes. Thus, in setting up a standard experimental procedure for the study of healing, a mechanically denuded surface was employed.

In guinea pigs weighing 350 to 450 grams, a central circular portion of skin and subcutaneous tissue, approximately 4 cm. in diameter, was excised from the midportion of the back, leaving the denuded fascial covering of the lumbodorsal muscles as the presenting surface. Human fibrinogen and thrombin were applied to the denuded surfaces in various forms and in combination with plasticizers and sulfadiazine. Animals used for control purposes received identical wounds which were permitted to form their own natural scabs. These scabs showed a tendency to crack earlier and more frequently than

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CALCULI REMOVED WITH COAGULUM

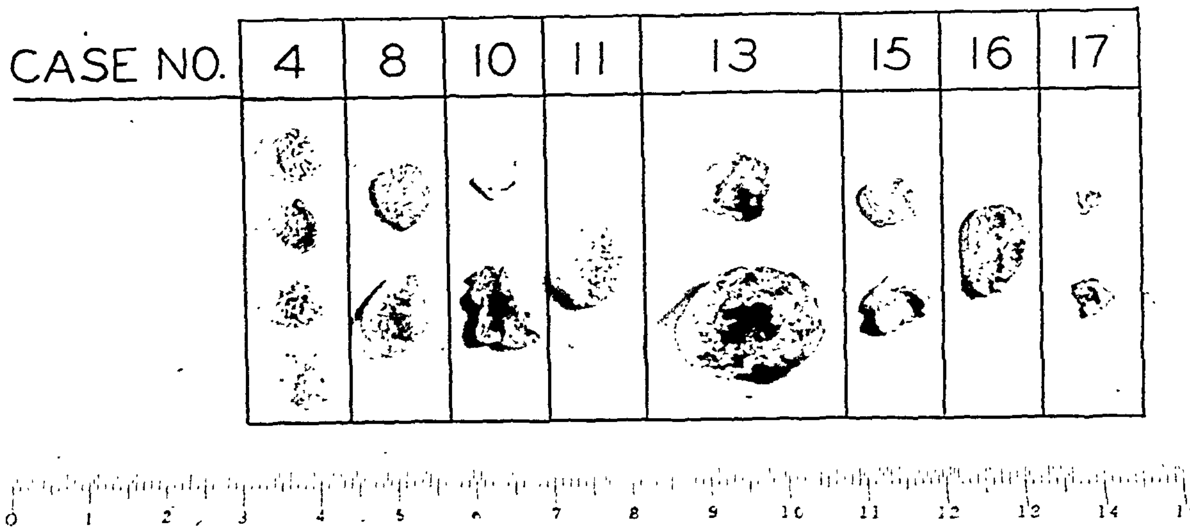

FIG. 1. PHOTOGRAPH OF RENAL STONES REMOVED BY MEANS OF COAGULUM

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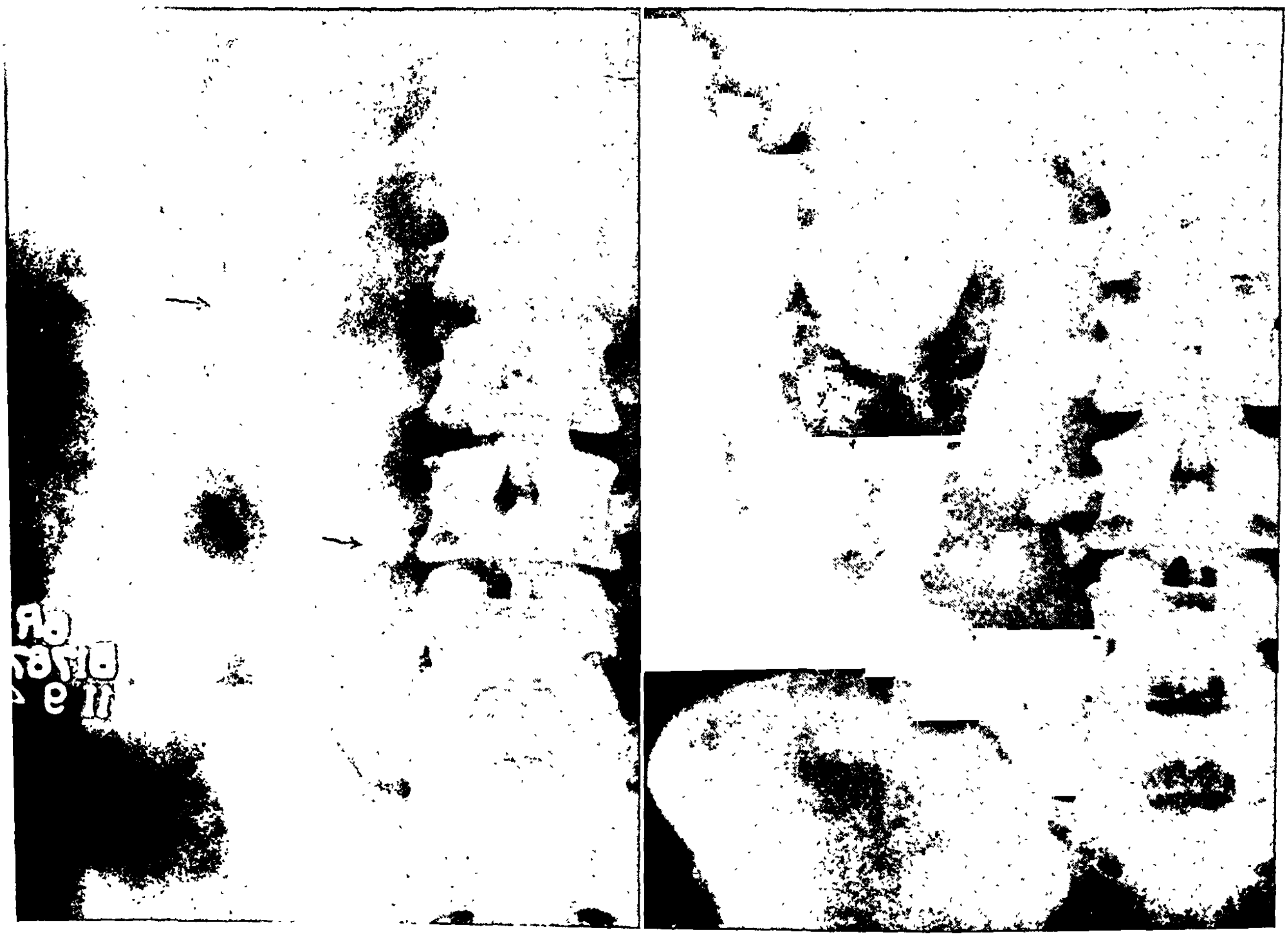

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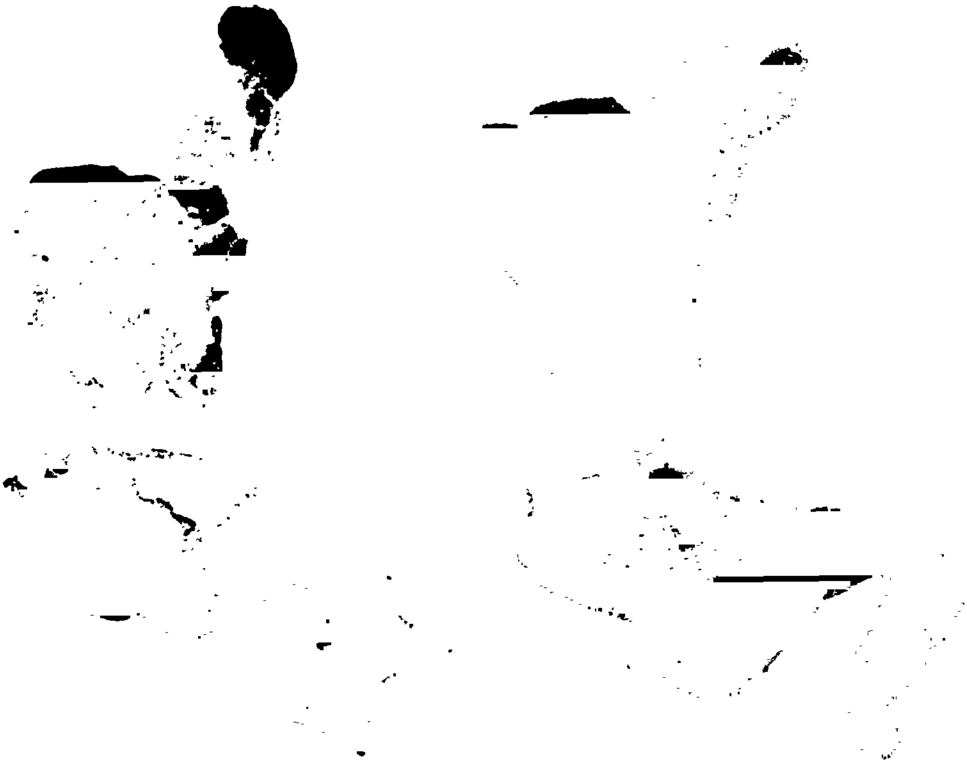

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FIG. 1. DRAWING OF GUINEA PIG 5 DAYS AFTER THE APPLICATION OF FIBRINOGEN-THROMBIN MIXTURE

The extent and placing of the denuded area used for experiments is indicated, as well as the appearance of the fibrinogen-thrombin eschar after 5 days.

did the applied films, with resultant secondary exudation.

In sections of wounds, 6 hours after the application of fibrinogen-thrombin mixtures, the applied film could be seen as resting on the tissue of the denuded surface and extending over the epidermal margins, thus providing an effective seal of the wound. Sections at 24 hours showed minimal hemorrhage and edema. Sections at 7 days revealed the repair process well under way. At the margins of the wound, the regenerating epithelium could be seen growing over the granulations and dissecting beneath the film (Figures 2 and 3). In instances where carbon particles had been incorporated in the films, only rarely was the carbon found in the underlying granulation tissue. Thus, the film remained almost entirely unorganized.

In the control series of untreated animals, the natural scabs showed a tendency to crack earlier and more frequently than did the applied films, with resultant secondary exudation. In general, histological sequences similar to those described in treated animals were observed. Although hemorrhage and edema were more frequent in the early stages, sections at 7 days demonstrated approximately the same amount of clear granulation tissue and the same amount of epithelialization at the wound margins.

These histological studies showed that the applied films were essentially inert. They evoked no inflammatory response in the denuded tissues. They fulfilled the function of a natural eschar in protecting the underlying wound during the repair process and permitting epithelialization to proceed at a normal rate.

Human studies. Certain of the fibrinogen-thrombin mixtures studied on guinea pigs were applied to a small group of burns, both of second and third degree, in human patients. Clinical observations served to indicate that in man as well as in guinea pigs there was no deleterious effect on the healing process.[6]

In the controlled study on the healing of skin graft donor sites, Cannon and Cope (1) reported a more rapid rate of epithelialization under fibrinogen-thrombin mixtures than under certain escharotics.

CLINICAL USE OF FIBRINOGEN-THROMBIN FILMS

In the studies recorded above, the films were made by clotting various types of mixtures of fibrinogen and thrombin on the site of injury. It became obvious from clinical experience with human burns that this procedure was too cumbersome and yielded mechanical properties unsuitable for use in the field. The development of

[6] We are indebted to Dr. Charles C. Lund, Dr. E. D. Churchill, Dr. Oliver Cope, Dr. Henry Marble, and Dr. Richard Wallace for their clinical material put at our disposal and for their aid and advice in the study of these patients.

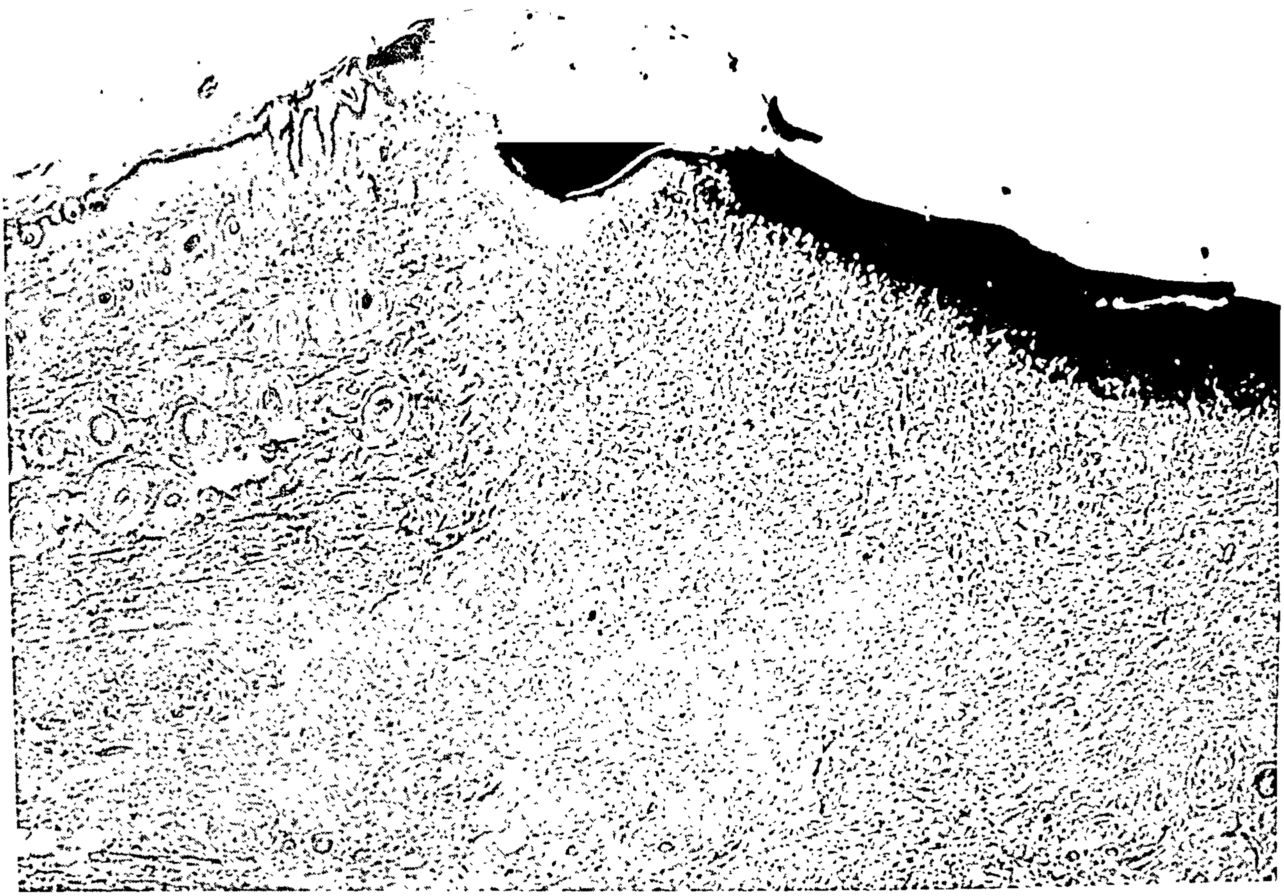

FIG. 2. LOW POWER PHOTOMICROGRAPH OF A DENUDED SURFACE COVERED BY FIBRINOGEN-THROMBIN MIXTURE FOR 7 DAYS

In the central portion (at the right of the illustration), there is granulation tissue covered by dark staining fibrinogen-thrombin mixture. The normal skin is shown on the left of the illustration. At the center is the epithelium which is extending over the defect and elevating the eschar.

preformed fibrin films of a wide range of properties by Ferry and Morrison (6, 7) opened the possibility of preparing a fibrinogen-thrombin dressing which would combine the lack of deleterious effect on the healing process with the properties considered above, namely, simplicity and speed in application and a range in mechanical properties permitting adaptation to many types of field conditions.

Films exhibiting high resistance to fibrinolysis proved most suitable for use in burns. Of these the following types (6) have been made available to us:

1. Type P: plain fibrin film.
2. Type F: fibrin film backed by an elastic cotton fabric similar in structure to "Ace Bandage."
3. Type W: fibrin film backed on one side by a waterproof plastic.
4. Type WF: fibrin film backed by an elastic cotton fabric whose outer surface is waterproof.

Preliminary trials of certain of these films have been made on a small group of burns at the Boston City Hospital.[7] These cases are presented in Table I. There are included both second and third degree burns of arms, legs, and trunk. On each patient, areas were treated with fibrin films and controlled by equivalent areas treated with vaseline impregnated gauze. In all cases, the dressings were quickly and easily applied. The relief of pain was rapid and consistent. In the case of the second degree burns, healing under the films was as rapid as the control areas in all instances. The fibrin films became lightly ad-

[7] We are indebted to Dr. Charles C. Lund and Dr. Stanley M. Levenson of the Burns Assignment of the Boston City Hospital for their clinical cooperation.

herent after application and so remained until epithelialization was complete, when the films would become dry and fall off. Dr. Lund, of the Burns Assignment, has stated that in second degree burns, "healing took place as rapidly as with any method known to date" (8).

In only one instance of second degree burns was there any infection; that was a staphylococcus albus infection which was not serious enough to delay healing.

In the third degree burns, the films appeared to be superior to the gauze ointment dressing used in control areas in that the film being only lightly adherent could be removed without pain or tearing underlying granulations, thus facilitating dressing in preparation of a sloughing area for grafting. When used over third degree burns, a moderate amount of fibrinolysis has been present after about the tenth day, which has been about the time when removal of the original dressing for study of demarcation of the slough is indicated. The fibrin films were not limited to the initial dressing but also used as secondary dressings.

COMMENT

Not only in the general management of the burned patient but also in the therapy of the injured surface, physiological and bacteriological considerations arise which are to a great measure independent of the actual material chosen for application, provided the material does not interfere with the normal process of repair. A large body of recent work has yielded striking advances in the elucidation of these considerations. To this aspect of the burn problem, the current observations made no contribution. It is essential, however, that the material for surface application be adaptable to the conditions of treatment which emerge from considerations of physiology and bacteriology.

In addition to lack of deleterious effect on the healing process, the fibrin films used in this study are adaptable to many programs of surface

FIG. 3. HIGHER POWER PHOTOMICROGRAPH TO SHOW THE EXTENSION OF THE EPITHELIUM UNDER THE ESCHAR

TABLE I

Cases of second and third degree burn surfaces treated with fibrin film

Case No.	Type film	Healing time		Infection	Debridement before dressing	Lysis of film
		Films (days)	Control (days)			
Second Degree Burns						
27	P	4	9*	None	****	0
29	P	6	6*	Staph. albus	None	0
30	P	4	4*	None	****	0
		9	9*	None	****	0
33	P	6	6*	None	None	0
35	P	5	5*	None	None	0
36	P	10	10*	None	None	0
37	W	10	**	None	None	0
Third Degree Burns						
31	P	Slough demarcated in 5 days	***	Pyocyan, β-Strept., Staph. aureus	None	3 per cent in 6 days
34	F	Slough well demarcated in 16 days	***	α- and β-Strept., Staph. aureus	None	40 to 60 per cent in 16 days
38	P	Slough well demarcated in 16 days	***	Not reported	****	Complete in 12 days

* Vaseline or boric ointment gauze.

** Control area covered with a cast which was not removed until after the sixteenth day.

*** In all third degree burns, the control areas were similar to the areas treated with the film except one instance where areas treated with Bentonite had an eschar-like crust which was removed with great pain. The removal of the vaseline gauze dressings on control areas was uniformly more difficult and painful than the removal of the films which was as a rule entirely without pain.

**** Loose skin removed, cleansed with soap and water, followed by saline irrigation.

therapy. Thus, either an open or closed dressing may be attained depending on the degree of adhesiveness of the film chosen and whether or not it be perforated. Again, films may be used without pressure, either in conjunction with a Koch pressure dressing (11) or beneath a case (10, 11). In point of fact, the Type WF film was developed in order to provide a speedy low bulk waterproof first aid dressing for the field wherein moderate pressure could be attained by reason of the elastic fabric backing.

In the light of recent studies (12), the evaluation of fibrin films has proceeded to date with bacteriostatic conditions uncontrolled. Sulfonamide drugs, penicillin, urea, methionine, or other substances of local value, may well be incorporated in films.[8]

Among the wide variety of films which have been employed on burned surfaces both by direct application and in the preformed state (2, 14 to 17), the only one in which the proteins involved in the natural coagulation mechanism were used is the fibrin membrane of Macfarlane (18). Whereas from the standpoint of healing, the membranes prepared by this worker were described as satisfactory, 3 drawbacks were listed. From the standpoint of mechanical properties, the membranes became brittle on drying and occasionally showed a tendency to lysis within 24 hours of application to a burned area. Their instability even under refrigeration did not permit transportation for field use. The method used for their manufacture involved the loss of the other parts of plasma, leading the author to remark, "It would probably be more economical

[8] In that recent evidence of Schmelkes and his coworkers (13) has suggested that sulfonamides buffered at high pH have a definite local action in the prevention of infection, further investigations are being carried out on films containing a high percentage of sodium sulfadiazine by Dr. William Andrus of the New York Hospital in a controlled series of experimental human burns.

to transfuse the patient with the plasma required to make the membrane."

The fibrin films we have used do not lead to the difficulties encountered by Macfarlane, in that when applied they remain flexible, they exhibit minimal tendency to fibrinolysis; packaged in sterile fashion they remain stable at room temperature for indefinite periods; the other components of the plasma from which they are manufactured are separately processed for their various therapeutic functions (4) which leaves the albumin available to "transfuse the patient."

It should be emphasized, in view of the reports of satisfactory use of many types of surface agents in burns, that a well-controlled comparative study is necessary to establish the respective merits of the various substances, from the standpoint of both civilian and military use (3). The observations here presented suggest that fibrin film can be adapted to the specifications, set forth at the initiation of this study, for the surface therapy of burns under certain field conditions.

SUMMARY

Observations of surgically denuded areas of animals and burns on humans are presented which suggest that human fibrinogen and thrombin mixtures have no deleterious effect on normal processes of repair. The use of preformed fibrin films, prepared from the proteins involved in the natural coagulation mechanism, is described in a small series of second and third degree burns. Such films are capable of adaptation to many programs of surface therapy. It is suggested that such films, particularly in the form of roll bandages, might prove a highly expedient fibrinogen-thrombin dressing for burns in the field, owing to simplicity and speed from the standpoint of application, and to lack of bulk from the standpoint of transportation.

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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

XX. THE DEVELOPMENT OF FIBRIN FOAM AS A HEMOSTATIC AGENT AND FOR USE IN CONJUNCTION WITH HUMAN THROMBIN [1,2,3]

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(Received for publication February 17, 1944)

The control of unusually rapid capillary oozing or of free venous bleeding where the site of hemorrhage is unsuitable for hemostasis by ligature, clip, or electrocoagulation has long been a problem in all fields of surgery. Many methods have been suggested to control such bleeding, but all have disadvantages which make them unsatisfactory in certain situations. This has been especially true in the field of neurosurgery.

The oldest and still probably the most commonly used method of controlling such bleeding is pressure applied with gauze sponges or cotton patties soaked in warm saline. This method, though often highly successful, is time consuming, and as Harvey (1) pointed out in 1918, and Putnam (2) has recently reemphasized, a tampon of this sort which must be removed will often drag the clot away from the bleeding point with recurrence of the bleeding. This fact has led to a search for a substance which would effect hemostasis and could be left *in situ* without exciting injurious tissue reaction.

First, the body tissues, fat, fascia, and muscle, were tried. Today the use of muscle, first introduced by Cushing in 1911 (3), remains the most satisfactory method to obtain hemostasis in difficult situations. Subsequent to 1911, Grey and Harvey (4, 5), both associates of Cushing, tried for the first time tampons made of both animal and human fibrin. Histological studies of absorption and resolution of implants of these materials in rabbits, cats, and dogs revealed less reaction than that occasioned by muscle implants. Blocks of sterile fibrin paper were found to be admirable hemostatic agents when used either alone or in conjunction with certain fluid clotting agents available at the time.

In the history of surgery many such fluid clotting agents have been used, ranging from the "Koagulen" of Fonio (6), prevalent in the second decade of this century, to the highly purified thrombins of animal origin (7 to 14) which have been developed within the last 10 years.

Putnam (2) has reported highly successful results using pledgets of soluble cellulose (15, 16, 17) with animal thrombin and later with thrombin of human origin, which became available in sufficient quantities for widespread clinical use in the course of large scale plasma fractionation (18, 19).

The latter program has, for the first time, made possible the achievement of an absorbable hemostatic agent, combining the functions of tampon and thrombin, which contains as components only human proteins involved in the natural clotting mechanism.

This material, known as fibrin foam, can be made with a wide range of physical and biological properties. It has been made in two general types: *One*, known as fibrin foam, contains a minimum amount of thrombin, is for

[1] This work has been carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[2] This paper is Number 25 in the series "Studies on Plasma Proteins" from the Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross.

[3] This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the United States Navy. The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

[4] Lieutenant, Medical Corps, United States Naval Reserve.

use with separately packaged thrombin; *the other*, made with thrombin intrinsically combined in it, needs no extra thrombin for hemostatic use. Ease of manufacture and clinical preference have dictated the selection of the separately packaged fibrin foam and thrombin as a primary standard for production. Fibrin foam has been extensively studied from the point of view of histological reaction, and has been successfully used in the clinic. These studies are reported in another paper of this series (20).

THE PROPERTIES OF FIBRIN FOAMS

The name "foam" derives from the fact that in the dry state the structure comprises dense strands of fibrin fibers, between which there are air spaces of macroscopic and microscopic size (Figure 1). The resultant product is thus of low density; the multiple channels within it permit penetration of fluids and give rise to an absorbent action. When dry, the foam appears as a homogeneous, dull, rough, slightly brittle, porous mass. As the moisture content increases, the foam loses its brittleness, becomes compressible, rubbery, slightly resilient, and when entirely wet there is some spontaneous shrinkage. These properties may all be varied by suitable alterations in preparation.

Range of mechanical properties. The two essential proteins, fibrinogen and thrombin, are separated in Fraction I and Fraction III-2, respectively, of the plasma fractionation process (18, 19). By varying the proportions of these constituents and the physical and chemical conditions under which foams are made, a wide range of mechanical and biological properties may be attained. At one extreme of this range is a light, fluffy, highly compressible product which wets with great ease and which, when wet, loses approximately 90 per cent of its dry volume through spontaneous shrinkage. Near this end of the range are foams best suited for attaining hemostasis of small persistent bleeders, such as are found on the dura. At the other end is a dense, firm, relatively less compressible product which wets slowly, and when wet, shrinks but 50 per cent of its dry volume. These types are more suitable for packing large cavities such as tumor beds.

While the mechanical strength of various types of foam depends upon conditions of their preparation, any given foam will show a variation in mechanical strength dependent upon its moisture content. The differences in mechanical strength, though not striking when the foams are dry, become pronounced as the moisture content is increased.

Further modifications of the physical properties of foams by chemical and physical treatment may be attained, notably in the direction of increased flexibility and elasticity. Such foams tend to regain their shape if a deforming force be removed, and thus do not lend themselves readily to moulding.

FIG. 1. FIBRIN FOAM IN THE DRY STATE

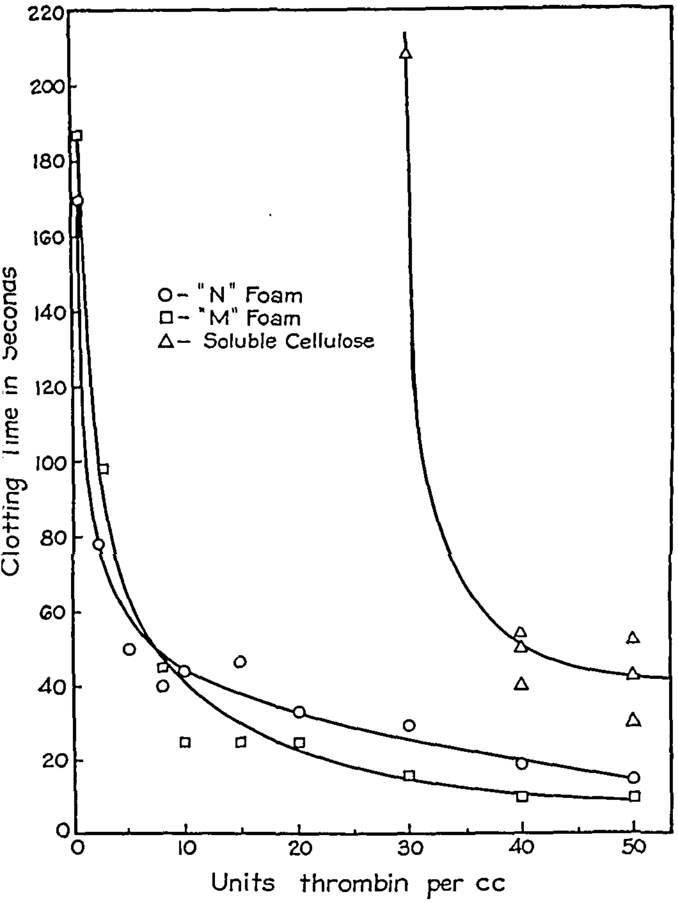

FIG. 2. *In Vitro* CLOTTING OF FIBRIN FOAM AND SOLUBLE CELLULOSE USED WITH THROMBIN SOLUTIONS OF VARYING STRENGTH

Thrombic activity. As in the instance of mechanical properties, a wide range of thrombic activity is attainable by varying the procedure of production. Fibrin foams of low thrombic activity, packaged as *fibrin foam*, are usually used with solutions prepared from separately packaged thrombin. *In vitro* or *in vivo*, the clotting time of a *fibrin foam* depends upon the strength of the thrombin solution used with it. This may be demonstrated by measuring the time required to clot 1 cc. of a standard fibrinogen solution with constant sized pieces of foam which are soaked in thrombin solutions of varying strength. Figure 2 shows this *in vitro* test applied to two kinds of fibrin foam, and soluble cellulose (supplied through courtesy of Dr. Tracy Putnam and Dr. Kenyon). Thus, foam would appear to be highly effective with weaker thrombin solutions than soluble cellulose.

If a foam is intended to be used alone as a hemostatic, the amount of thrombin incorporated in it will determine its *in vitro* and *in vivo* clotting times. With any given foam of this type, the *in vitro* and *in vivo* clotting times will vary with moisture content, the length of time it soaks in saline, and the volume of solution.

Curve 1, Figure 3, is the *in vitro* clotting time of constant sized pieces of foam when soaked in an infinite excess of saline, while curve 2 measures the effect of soaking the foam in just slightly more saline than the foam could absorb. In both cases, the clotting time reaches a minimum when the foam is first completely wet, but with an infinite amount of saline the thrombin continues to diffuse out and the clotting time rises, while with a small amount of saline the thrombin diffusion soon reaches equilibrium and the clotting time becomes constant.

Fibrinolysis. In that the source materials for production of foam, Fraction I and Fraction III-2, both contain fibrinolytic activity, the rate of lysis of the wet foam *in vitro* will be in the main a function, first, of the concentration of this activity, and secondly, of the susceptibility of the fibrin. Both of these factors may be altered by appropriate measures. Thus, it is possible to prepare a foam which when wet will lyse spontaneously with considerable speed *in vitro*, which is readily susceptible to bacterial fibrinolysin and which disappears relatively rapidly when implanted in tissue. On the other hand, it is possible to produce a foam, with the natural fibrinolysin inactivated, whose fibrin is only slightly susceptible to bacterial fibrinolysins.

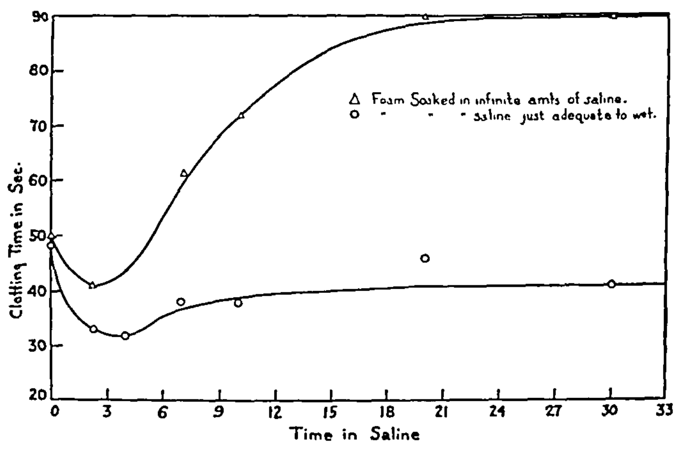

FIG. 3. EFFECT OF SOAKING FIBRIN FOAM THROMBIN IN VARYING AMOUNTS OF SALINE ON CLOTTING TIME

Curve 1 (△) Curve 2 (O).

In this case, both lysis *in vitro* and disappearance in tissue are slow.

Sterilization of fibrin foams. The fibrin foams released for clinical use are sterile. In that resterilization by boiling or by the autoclave seriously changes the mechanical properties, the thrombic activity, and the histological sequences following implantation, the unused material in an opened package should be discarded.

Incorporation of anti-bacterial agents. Antibacterial agents may be used with fibrin foam whenever they are indicated. These agents may be incorporated in the foam, put up with the thrombin, or mixed at the time of use.

COMMENT

Fibrin foam is one of a group of fibrous structures of potential hemostatic value which can be prepared from the products of human plasma fractionation. From the range of properties attainable in this group of structures, it becomes the province of the surgeon to chose that combination which is best suited to his particular problem.

The clinical and pathological studies reported in subsequent papers have been carried out on a group of foams which, in physical characteristics of porosity, compressibility, and strength, are near the light, fluffy end of the possible range. From the standpoint of bacterial fibrinolysis, the natural enzyme has been inactivated and the fibrin rendered resistant. Fibrin foam (low thrombin) has been used in conjunction with separately packaged thrombin.

Whereas this type of matrix would seem peculiarly adapted to and has, at present, been most used in neurosurgery, it may well emerge that an entirely different structure would be required should these products prove of value for other situations; for example, in the control of oozing as presented by chest wounds, injuries to the liver, and gynecological surgery.

SUMMARY

A fibrous protein matrix of a wide range of mechanical and biological properties has been prepared from the human plasma proteins involved in the natural coagulation mechanism. This matrix is designed to combine the function of an absorbable tampon with thrombin activity for use in those instances of hemorrhage where conventional surgical methods of hemostasis are not entirely satisfactory.

Fibrin foam for use with thrombin solution has been the first of these to be available for widespread clinical use. Its structure and characteristics are considered in detail.

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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

XXI. THE USE OF FIBRIN FOAM AS A HEMOSTATIC AGENT IN NEUROSURGERY: CLINICAL AND PATHOLOGICAL STUDIES[1,2]

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(Received for publication February 17, 1944)

In neurosurgery, there are several types of bleeding for which the hemostatic agents now in general use are not satisfactory. By far the most common is capillary oozing. The control of such oozing in the dura, brain, spinal cord, and especially the beds of intracranial tumors, frequently prolongs neurosurgical procedures and at times is difficult, if not impossible, to stop completely. Another type of bleeding for which the common hemostatic agents are not satisfactory is that from the dural sinuses and other large venous channels where ligature cannot be applied. The various substances which have been employed in these situations up to the present time have been discussed elsewhere (1, 2).

When fibrin foam was prepared by Bering (1) from the products of large scale plasma fractionation (2, 3), the investigation of its uses in neurosurgery was undertaken, along with a study of the tissue reactions to its presence. There are now available for evaluation both clinical material and a series of experimental studies in monkeys.

CLINICAL MATERIAL

Fibrin foam and thrombin have been used for the control of bleeding in 170 neurosurgical patients at the Peter Bent Brigham Hospital and The Children's Hospital, Boston.

The thrombin is dissolved in 30 cc. of saline solution at room temperature and foam soaked in this solution a few minutes before use. The technic of application to the bleeding point necessarily varies with the circumstances. In general, the area has been dried by suction, the moist foam applied, and a cotton pattie held over the foam until it has become adherent. The foam, applied in this way, has proved to be very effective in controlling oozing from the outer surface of the dura, bleeding from the substance of tumors, and from the tumor bed after removal, as well as from larger vessels, such as large superficial cortical veins and even from dural sinuses.

The material has been left in place in all of these patients in amounts varying from small fragments to a mass the size of a golf ball. In no instance has there been any clinical evidence of cortical irritation. Furthermore, in those patients in whom the operative field was seen after 14 hours to 81 days, either at secondary operation (15 patients) or at autopsy (4 patients), there was no evidence of an inflammatory reaction or other unfavorable result.

The following case history is given to illustrate its use:

A 36-year-old housewife was admitted October 16, 1943 because of failing vision. Two years and four months previously glasses had been fitted but she was not aware of loss of visual acuity until 5 months ago. There had been a mild personality change, of which the patient was not aware, through a 2-year period. Mild headaches had been associated with menses for many years. Examination showed almost total loss of vision in the right eye and ability to count fingers at one foot with the left eye. Roentgenograms revealed erosion of the sella turcica and slight thickening of the right frontal bone. A diagnosis of meningioma was made and a right frontal bone flap was turned down. The tumor involved the frontal bone and

[1] This work has been carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[2] This paper is Number 26 in the series "Studies on Plasma Proteins" from the Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross.

dura down to and into the right frontal sinus. The involved bone and dura were resected and the large vessels around the tumor clipped or coagulated. A mass filling the entire fossa and displacing the falx to the left of the midline was then mobilized and lifted out. A large ball of foam was immediately placed in the tumor bed which was bleeding freely from numerous points. This was held in place with a piece of lintine (cotton) for several minutes and the central portion of the foam mass then pulled gently out leaving a thin shell of the material lining the bed. At this point there was no bleeding. The cavity was filled with saline, the dural defect replaced with fibrin film (described below) and the flap closed. Recovery was uneventful. Histological diagnosis: Meningioma.

(Note: There is no reason why the entire mass of foam should not have been left in place if necessary. The use of the foam made it possible to remove the entire tumor at one time and to leave the tumor bed entirely free from oozing. This was, moreover, accomplished in a much shorter time than would have been possible without the use of the foam.)

TISSUE REACTIONS OF FIBRIN FOAMS IN HUMAN PATIENTS

Fibrin foams before implantation. Samples of fibrin foams were fixed in Zenker's fluid, embedded in paraffin, cut, and stained with phloxine-methylene blue. Under these circumstances, the foam was found to consist of a network of dark blue fibers with clear interstices. This is a two-dimensional picture; in three dimensions, the structure of the foam would be more like a honeycomb. There was a close similarity in staining reaction between the fibers of the foam and the fibrin of spontaneous blood clots. However, the fibers of the foam were coarser and more homogeneous than were the fibers of freshly clotted blood.

Tissue reactions in human patients. We have

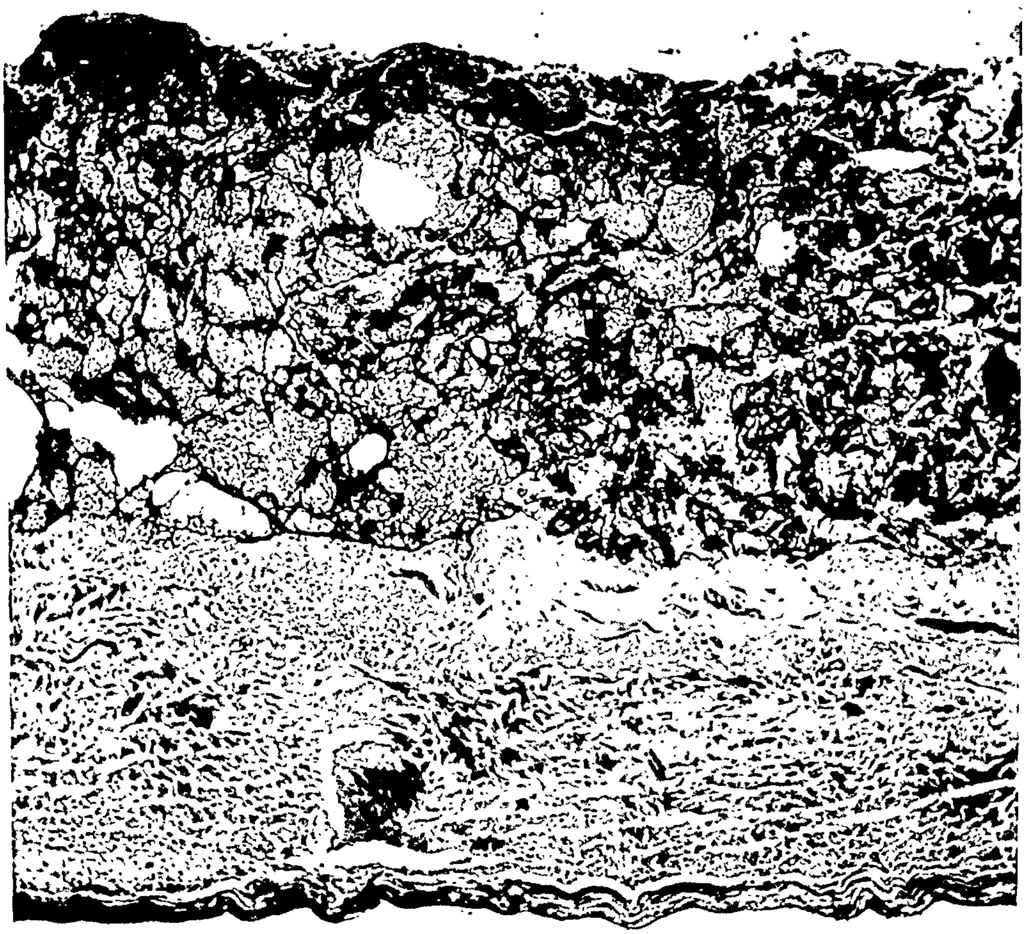

FIG. 1. LOW POWER PHOTOMICROGRAPH SHOWING FIBRIN FOAM ON THE SUPERIOR SURFACE OF THE DURA

The specimen was obtained at autopsy, 14 hours after operation. The illustration shows the histological appearance of the foam and the lack of immediate reaction in this patient.

FIG. 2. PHOTOMICROGRAPH SHOWING A SMALL FRAGMENT OF FOAM IN A SPECIMEN OBTAINED AT SECONDARY OPERATION 24 DAYS AFTER THE ORIGINAL OPERATION

This was the only fragment of foam identified.

had the opportunity to study the tissue reactions to fibrin foams in 19 human patients. In these patients, foams were removed at secondary operations or (in 4 instances) at autopsy. The length of time that the foam had been in contact with the tissues varied from 14 hours to 81 days. Figure 1 shows the appearance of foam, which had been applied to the dura at operation, 14 hours before death. The appearance of the fibers of the foam was essentially the same as before it had been in contact with the tissues. The interstices, however, had become filled with clotted blood. There was no reaction in the tissues. The complete absence of tissue reaction in this specimen may be due in part to the poor general condition during the 14 hours that the foam was in contact with the dura.

In a specimen of foam obtained 3 days after it had been left on the dura, the total amount of foam was much less than that originally placed there at the time of the operation but considerable numbers of fibers were easily identified. The foam became eosinophilic at the periphery where it was in contact with living tissues; except for this narrow zone, the foam retained the same appearance as it had before it was placed in the patient. About the foam, there were moderate numbers of polymorphonuclear leukocytes and lymphocytes. Also present were scattered mononuclear cells which contained homogeneous eosinophilic material, judged to be minute fragments of foam.

No large fragments of the foam were found in any of the specimens except those recovered at 14 hours and at 3 days. In specimens obtained from 7 to 24 days after they had been left in place at operation, either a few very small fragments were present or no trace of foam was found.

Figure 2 illustrates the only remnant of the foam which could be identified 24 days after a large amount had been used to control oozing from the dura. There was a small mat of fibrous

tissue about the fragment and a few mononuclear cells were seen. The material about the fragment was mostly fresh blood from the second operation at which the tissues were removed. Since the foam was used to cause the coagulation of blood, it was inevitable that the foam should at once be surrounded by blood clot. There would be a considerable number of fibroblasts produced in the organization of the blood clot. Hence, it was impossible to determine how many of the fibroblasts were due to the presence of the foam and how many to the blood clot. It appeared from this and other specimens, that the tissue reaction to the foam was less than to a blood clot of comparable size.

Figure 3 shows a portion of cerebral cortex on which fibrin foam had been used as a hemostatic agent 81 days previously. No trace of foam remained. While the fibrous tissue of the arachnoid was somewhat increased, this was not in excess of that expected from the operative procedure alone. There were no tissue changes which could be definitely related to the fibrin foam anywhere in the specimen.

ANIMAL EXPERIMENTS

A series of animal experiments was also carried out to determine the local reaction of cerebral tissues to foam and thrombin. For this study, 37 monkeys (*Macaca mulatta*) were used to test 6 standard conditions. These not only provided an opportunity to study the tissue reactions to fibrin foam but also included an opportunity to compare the tissue reaction due to foam with that due to soluble cellulose. In each animal,

FIG. 3. PHOTOMICROGRAPH OF MENINGES AND CEREBRAL CORTEX SHOWING THE SITE AT WHICH FIBRIN FOAM HAD BEEN PLACED 81 DAYS PREVIOUSLY. NO RESIDUAL FOAM WAS IDENTIFIED.

While the fibrous tissue of the meninges was increased, the amount was not in excess of that which could be accounted for by the operative procedure alone.

bilateral craniotomies were performed, the dura opened and the parietal lobe exposed. Foam and thrombin were placed (*1*) on the normal cortex, (*2*) on cortex damaged by multiple needle wounds which produced bleeding, and (*3*) within the substance of the parietal lobe at a depth of 3 to 4 mm. Since it would obviously be desirable to use the material in compound head injuries with gross contamination, particularly under war conditions, the same procedures were carried out using foam and thrombin in combination with sulfadiazine and again with penicillin. The animals were sacrificed at periods varying from 3 hours to 3 months. There was no physiological evidence of cortical irritation or other untoward effects in any of these animals.

When the monkeys were sacrificed, the skulls were fixed in 10 per cent formalin after excess tissue had been trimmed away. The skull was then dissected away so that the relationship of meninges and cerebral cortex could be studied without distortion. Blocks of tissue were then cut, embedded in paraffin, and stained with hematoxylin and eosin.

The earliest tissue reaction in the meninges was the appearance of small numbers of mononuclear cells and polymorphonuclear leukocytes. This was followed by a rapid disappearance of the foam with condensation into a more compact mass. The cellular infiltration, never extensive, became minimal and there was a slight proliferation of fibrous tissue. Only very small bits of foam were present after 1 week and no fragments at all could be identified at 3 weeks.

When relatively large pieces of fibrin foam (4 × 4 × 6 mm.) were inserted directly in the substance of the cerebral cortex, these disappeared at least as rapidly as on the meninges. The maximum tissue reaction noted in any of these monkeys was a slight gliosis at the site of implantation of the foam.

Some of the experiments were planned to provide a basis for comparison of the tissue reactions of fibrin foam and of soluble cellulose soaked in human thrombin solution. All the monkeys in this group had identical operative procedures on each side of the skull as outlined above, but fibrin foam was implanted on one side and soluble cellulose soaked in thrombin on the other. In 2 monkeys, there were adhesions between the arachnoid and dura on the side on which soluble cellulose soaked in thrombin had been placed but no adhesions on the opposite side, where fibrin foam had been implanted. The extent of tissue reaction and rate of absorption were otherwise quite similar with the two hemostatic agents.

When sulfadiazine was placed in experimental wounds containing fibrin foam, there were no detectable changes in the physical characteristics of the foam or in the character or extent of the tissue response. In experimental wounds containing both fibrin foam and penicillin, the properties of the foam were unchanged and the tissue response was not altered.[3]

COMMENT

From the clinical point of view, fibrin foam has proved to be a valuable hemostatic agent in neurosurgical procedures. It is readily prepared from the dry state in the operating room and the thrombin dissolves quickly. Very little delay, therefore, is occasioned in preparing the material even when vigorous bleeding is encountered unexpectedly.

By the use of the foam and thrombin, as outlined in preceding paragraphs, complete control of oozing is secured. This applies not only to bleeding in such situations as the dura where the more conventional methods are adequate but time consuming, but also to such locations as the beds of neoplasms, where the conventional agents are not always satisfactory. It is also of great assistance in controlling more vigorous bleeding from large venous channels, such as the dural sinuses and cerebral veins. Fibrin foam, however, is seldom effective in the management of bleeding from large arteries.

The material has a conspicuous advantage over muscle in that it could be made easily available in any desired quantity and in that it causes less tissue reaction than does muscle.

In this series of cases, the foam has frequently saved considerable time and has made it possible to accomplish complete extirpation of a tumor which might otherwise have been impossible or

[3] The penicillin was provided by the Office of Scientific Research and Development from supplies assigned by the Committee on Medical Research for clinical investigations recommended by the Committee on Chemotherapeutic and Other Agents of the National Research Council.

would have had to be divided into two or more stages.

The tissue reactions to the foam are minimal but not entirely absent. Not only are they slighter than the reaction caused by human muscle when used as a hemostatic agent, but they are also considerably less than those caused by silk and catgut sutures or bone wax. The amount of fibrous tissue produced is more nearly comparable to that resulting from the organization of a small blood clot. As a matter of fact, it is difficult to determine whether the small amount of fibrous tissue left at the site of implantation of foam is due to the foam itself or to organization of the blood clot which the foam has induced. It is possible that these minimal tissue reactions may be further reduced by changes in the methods of preparation of the foams so that they are absorbed even more quickly.

The experimental evidence indicates that the foam may be used in wounds treated with sulfadiazine and penicillin without change in tissue reaction. Clinical experience in a few instances is in accord with the experimental studies on this point.

Additional illustration and discussion of the utility of fibrin foam has been presented elsewhere (1, 4).

SUMMARY

Fibrin foam has been tested under a variety of experimental conditions in a series of monkeys. The tissue reactions were minimal in all animals, including those in which the foam was implanted in conjunction with sulfadiazine and penicillin.

The foam was used in 170 neurosurgical patients, under varying conditions. Its rapid hemostatic action has shortened many operations. In some instances, it has made successful operation possible when other hemostatic agents would have been inadequate.

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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

XXII. FIBRIN FILMS IN NEUROSURGERY, WITH SPECIAL REFERENCE TO THEIR USE IN THE REPAIR OF DURAL DEFECTS AND IN THE PREVENTION OF MENINGOCEREBRAL ADHESIONS [1,2]

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The problem of repairing dural defects and of preventing adhesions between damaged cerebral cortex and adjacent tissues has never been satisfactorily solved. The question is particularly pressing at this time because of the large number of head injuries in combat which result in loss of dura and cerebral tissue. The variety of substances now in use by neurosurgeons provides good evidence that none of them is completely satisfactory. Those most widely employed have been fascia lata transplants, gutta percha sheets, preserved dura, rubber, amniotic membrane, and metals. The use of some one of these has generally been considered preferable to leaving a dural defect, although in some cases it is probable that the dural substitute is responsible for the subsequent convulsions, rather than the gliosis following or due to the injury itself.

Experimental and clinical data are presented which suggest strongly that the best solution to date for these and related neurosurgical problems is offered by the fibrin films prepared by Ferry and Morrison (1, 2) from the products of large scale plasma fractionation (3, 4).

ANIMAL EXPERIMENTS

In order to determine what irritative effect the film might have on the normal cerebral cortex, it was implanted as a dural substitute in 6 large monkeys (*Macaca mulatta*) under nembutal anesthesia. A midline incision was made in the scalp and the soft tissues reflected laterally. On the right side, an osteoplastic bone flap was turned down; on the left side, the bone was removed with rongeurs in an area corresponding in size to the bone flap (roughly 2 × 3.5 cm.). The dura was removed throughout the extent of the bony opening and a piece of film, slightly larger than the defect, left *in situ*. The edges of the film were placed under the cut edge of the dura without suturing. Following this procedure, the bone flap was replaced on the right, the temporal muscle sutured over the defect on the left where a similar piece of film had been placed, and the scalp closed. The procedure was repeated in other monkeys with the following variations: (*a*) the cortex was traumatized by multiple needle punctures producing hemorrhages; (*b*) sulfadiazine was placed on both the normal and traumatized cortex before the film was implanted. These animals were observed for periods varying from 24 hours to 6 months. During this period, they were entirely free from any signs of cortical irritation or other physiological abnormalities. The monkeys were then sacrificed with an overdose of nembutal, the heads were removed, and excess tissue trimmed away. The skulls were fixed in 10 per cent formalin for 10 days in order to allow dissection of the area of operation without distortion of relationships. At the end of the period of fixation, the skull was carefully removed without opening the dura. After gross studies had been completed, blocks were selected, embedded in paraffin, and the sections stained with hematoxylin and eosin.

When the brains were studied in the gross, it was found that the films persisted for considerable periods of time. A monkey sacrificed 6 months after the implantation of film was particularly significant (Figure 1). In this animal, there were no adhesions between the cerebral cortex and the film on either side. The membrane had become incorporated as an integral part of the dura with fusion between the edges of the dura and the film so that the whole formed a continuous sheet. Figure 1 shows clearly the

[1] This work has been carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[2] This paper is Number 27 in the series "Studies on Plasma Proteins" from the Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross.

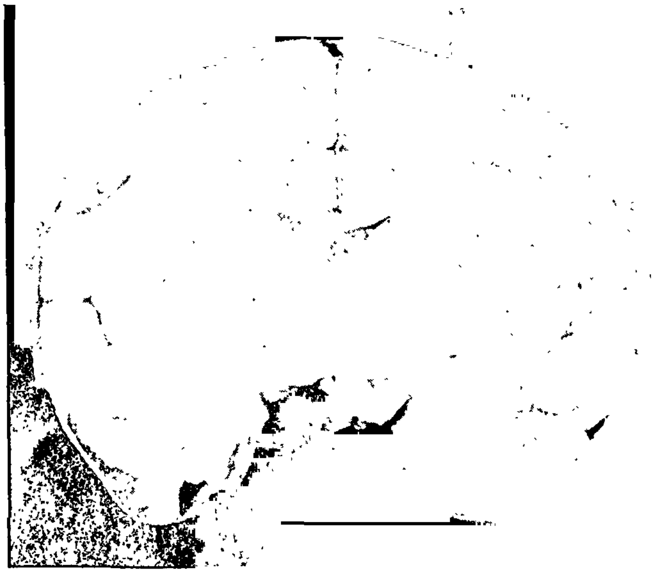

FIG. 1. CROSS SECTION OF MONKEY'S BRAIN SACRIFICED 6 MONTHS AFTER FILM WAS LEFT OVER THE CEREBRAL CORTEX TO CLOSE A DURAL DEFECT.

Notice that the film is still present. There are no adhesions between the cerebral cortex and the implanted film. The cerebral cortex appears entirely normal.

complete absence of adhesions and the normal condition of the underlying brain. Other monkeys sacrificed at shorter periods were comparable to this one.

In order to obtain a satisfactory base-line for microscopical studies, some fibrin films were fixed in formalin before implantation and stained with hematoxylin and eosin. Under these circumstances, the fibrin films appeared as uniform pink staining sheets without any trace of fibrillar structure.

In the monkeys sacrificed a short time after the films were implanted, there was a very slight infiltration with mononuclear leukocytes at the periphery of the films. Giant cells at this stage were entirely absent. The hematoxylin and eosin stain showed the same pink color of the films whether the films were in contact with living tissue or not. This was in contrast to the findings in sections stained with phloxine and methylene blue, described in the section of this paper devoted to fibrin films in human patients. The difference was regarded as dependent upon the staining technic rather than species difference. In monkeys sacrificed after 3 to 6 months, the films had become thinner and in some instances were invaded near the point of dural attachment by strands of fibrous tissue. Under these circumstances, occasional giant cells made their appearance about isolated bits of film but there was no striking giant cell response.

When sulfadiazine was applied at the same time that the films were implanted, there was no change in the character or extent of the tissue reactions. No adhesions resulted.

CLINICAL USE OF FIBRIN FILMS

Fibrin films have been used to repair dural defects or placed between cortex and overlying damaged tissue, muscle, or bone in 59 patients. The material has been recovered at secondary operations in 18 instances. In the first few patients, it was used only where there were unusually large dural defects leaving the brain unprotected. In one such case, the film was recovered and replaced by a second one, as described in the following case history:

Case 1. An extremely ill 2-year-old child entered the hospital because of drowsiness and vomiting for 12 days. There was a very strong history of pica involving lead-containing paint. Physical examination indicated a pale, comatose child with right internal strabismus, marked papilledema, and cracked pot sound. Roentgenograms showed separation of the coronal sutures and typical "lead lines" in the long bones. On lumbar puncture, the cerebrospinal fluid pressure was found to be over 700 mm. of water; the total protein was 204 mgm. per cent. A diagnosis of lead encephalopathy was made and decompression done. This was accomplished by turning down an unusually large right fronto-temporo-parietal bone flap and removing the temporal bone with rongeurs. The dura was then opened widely and the ventricle tapped (spinal drainage was also continued throughout operation). The brain protruded markedly but without damage to the cortex. The large dural defect was covered with a sheet of fibrin film roughly 10 × 12 cm., the bone flap left out, and the scalp closed. A week later the same procedure was performed on the left, except that on this occasion, it was possible to replace the bone flap. Eighteen days after the original operation the scalp was reflected in order to replace the first bone flap. It was thus possible to examine the original film *in situ*. There were no adhesions whatever between the film and overlying muscle or between film and cortex. This film was removed and replaced by a second similar film in order to carry out histological studies. The patient has made a very satisfactory recovery; cerebrospinal fluid pressure is normal and she has been symptom-free for one year.

Having recovered the film at reexplorations in several other patients, its use was extended to include defects which would ordinarily be covered by muscle. This group includes several sub-

occipital explorations, subtemporal decompressions, and laminectomies in which the film was left between the central nervous system tissue and adjacent muscle. The following case history will illustrate one such instance:

Case 2. A 2-5/12-year old boy was admitted with complaint of disturbance in gait. Physical examination showed signs of cervical cord compression. Lumbar puncture revealed evidence of block in the circulation of cerebrospinal fluid. On July 12, 1943, a laminectomy was performed with exposure of the spinal cord, which was markedly enlarged. A dorsal incision was made in the cord and, at a depth of 2 mm., a soft reddish-brown tumor was encountered. The incision of the cord was extended to the limits of the enlargement and a small fragment of tumor removed for histological study. A piece of fibrin film was then placed over the protruding tumor and spinal cord, the dura left open, and muscles sutured. The tumor proved to be a cellular astrocytoma. The wound was reopened 7 days later, at which time the film was easily removed, since it was not adherent to the spinal cord, tumor, or adjacent muscle. The tumor had largely extruded itself and could be almost entirely removed. It was felt that the local protection of the tissues by the film greatly facilitated the second stage. In order to leave as much room as possible in case of recurrence, the dura was again left open and a second piece of film left over the cord. At the present time, there is no perceptible interference with function.

Material for study of the tissue reactions of fibrin films in human patients was available in 18 instances. These films were removed at secondary operations from 14 hours to 81 days after the film had been left in place. Blocks were fixed in Zenker's fluid, embedded in paraffin, and the sections stained with phloxine and methylene blue. Films prepared for study by this technic, without implantation, were seen on microscopical study as uniform deep blue sheets. In the specimen obtained at 14 hours, the only change noted was the slight alteration in staining reaction at the periphery where the film was in contact with the tissues of the patient (Figure 2). This portion took the phloxine stain for a very narrow zone where the film was in contact with living tissue, while all the rest was uniform deep blue. In specimens removed after longer intervals, the same staining reaction was present,—the film was deep blue where not in contact with the tissues and red for a narrow zone where it was. This difference in staining reaction was not apparent when the tissues were stained with hematoxylin and eosin.

FIG. 2. LOW POWER PHOTOMICROGRAPH OF A FILM WHICH HAD BEEN IN PLACE OVER THE CEREBRAL CORTEX OF A HUMAN PATIENT FOR 14 HOURS

The homogeneous character of the film is indicated.

In specimens obtained 3 to 19 days after the films had been left in place, there was some aligning of fibrous tissue along the periphery, together with a slight infiltration with mononuclear cells and lymphocytes but polymorphonuclear leukocytes were rare. At the longer intervals, some infiltration of the film by connective tissue was noted. The specimens obtained after 81 days showed a continuation of these processes. There was a layer of fibrous tissue about the same width as the original film. Within this layer, there were scattered masses of fragmented film still present. About these bits of film were a few mononuclear cells and lymphocytes, and occasional giant cells were found about minute fragments of the film.

COMMENT

The problem of providing a dural substitute and of introducing a protective layer between tissues of the central nervous system and adjacent mesenchymal tissue is an extremely difficult one. Any material offered as a solution must be subjected to critical examination over a long period of time. The fibrin film used in the above animal experiments and in clinical problems would thus far seem to have distinct advantages over the substances previously available. It is transparent, flexible, easily cut, and can be placed to conform with a rounded surface. With reasonable care, it can be sutured to the surrounding dura or simply pressed under its free margin. If secondary exploration should be necessary, the film can be readily stripped out from the place where it was left, thus facilitating the identification of layers and shortening the

operative procedure. In addition, the film has whatever advantages may be attached to materials of human origin. There has been no physiological evidence of irritative phenomena either in the experimental animals or in the human patients. The gross anatomical study emphasizes the lack of adhesions between the film and adjacent tissues. The histological study did not reveal any contraindication to the use of this material. When sulfadiazine was placed in wounds where film had been implanted, the properties of the film were not altered and no irritative phenomena resulted. The gross and microscopical appearance after intervals were comparable to those in wounds in which film was implanted without sulfadiazine.

The films used in the patients reported in this paper have all been of the type which has been described by Ferry and Morrison (1). This is a tough and rubbery film which seems to possess the physical properties desirable in a dural substitute. However, the physical properties of the fibrin films can be varied over a considerable range by small alterations in the technic of their preparation. Should subsequent experience indicate that different physical properties were preferable in certain locations, films with these characteristics could be readily prepared.

From the information at hand, the fibrin films would seem to be definitely superior to metal foil, preserved dura, fascia lata, rubber, gutta percha, or amniotic membrane.

Additional illustration and discussion of the use of fibrin films has been presented elsewhere (5).

SUMMARY

Fibrin films have been tested as a protective layer for the central nervous system in a series of monkeys and patients.

These materials have been found very satisfactory for the repair of dural defects and the prevention of adhesions between damaged nervous tissue and adjacent structures.

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CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

XXIII. THE EFFECTS OF FEEDING POSSIBLE BLOOD SUBSTITUTES ON SERUM PROTEIN REGENERATION AND WEIGHT RECOVERY IN THE HYPOPROTEINEMIC RAT [1]

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Because of disadvantages connected with the use of the hypoproteinemic dog for the evaluation of the biological potencies of *small* quantities of dietary proteins, we have utilized the hypoproteinemic rat; the present report describes experiments in which proteins of possible value as blood substitutes were fed to protein-depleted rats in order to ascertain their abilities to promote serum protein regeneration and weight recovery.

The method is, in principle, a modification of that used by Weech and others (1, 2). Instead of measuring only albumin regeneration, however, it determines regeneration of total serum protein. As we have used it thus far, the method may be summarized as follows: Healthy adult male albino rats are placed on a low-protein, low calorie diet of a protein content usually varying between 1.75 and 2.0 per cent (N $\times$ 6.25). The composition of the diet (3E) (for 10 kilograms) is as follows:

Carrots, finely ground, uncooked	3000 grams
Ruffex (Fischer)	500 grams
Lard	400 grams
Corn starch	4400 grams
Water	1000 grams
Salt mixture (3)	400 grams
Brewer's yeast	200 grams
Liver concentrate (Wilson and Company, 20 : 1)	100 grams
Choline chloride	10 grams
Oleum percomorphum	16 drops
Calcium pantothenate	20 mgm.
Pyridoxine hydrochloride	20 mgm.
Riboflavin	50 mgm.

In making up the diet, the dry ingredients are weighed and thoroughly mixed in a large rod mill, after which the melted lard containing the oleum percomorphum is added and thoroughly mixed into the main mass. Then the carrots and the water containing the dissolved purified vitamins are added and the mixing continued. Each mixing procedure takes about 15 minutes. The diet yields approximately 2.3 calories per gram. When 18 to 22 per cent of vitamin-test casein (Smaco) is substituted for an equal weight of corn starch and fed on an average daily intake of approximately 20 grams per rat, the rats gain weight and appear healthy. The vitamin supplements with respect to vitamin A, thiamin, riboflavin, pyridoxine, pantothenic acid, and vitamin D are well above those commonly considered as daily requirements for the rat. No additional ascorbic acid or niacin have been provided, as the rat has not been found to require them. In order to ensure an adequate supply of choline above the amount occurring in carrots, 0.1 per cent has been added to ration 3E.

METHOD OF DIETARY ASSAY

Healthy adult male albino rats, ranging between 200 and 300 grams in weight and of as nearly uniform size and age as possible, are weighed, ear-marked, and placed 6 to a cage in rabbit cages with wire-screen floors. One rat from each group is usually bled from a tail-vein for a total protein determination, after which most of the groups are fed a low protein, low calorie ration (2E or 3E) for 10 to 12 weeks, at an average daily intake of 20 grams per rat per day. A few groups are fed similar quantities of the control diet, *i.e.*, the low basal ration with from 18 to 22 per cent of vitamin-test casein (Smaco) substituted for an equal weight of corn starch. These animals serve as controls for later experiments on antibody-production and chemical fractionation of the livers; they also supply a group of well-nourished animals with which to compare the regeneration levels of weight and serum protein which the depleted rats attain after having been fed one of the test-proteins. All animals of both groups receive water *ad libitum* and about 15 to 20 grams of leaf lettuce per rat per week.

[1] The products of plasma fractionation employed in this work were developed by the Department of Physical Chemistry, Harvard Medical School, Boston, Massachusetts, under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University. The fractions of bovine plasma were prepared at the Armour Laboratories, Chicago, Illinois, under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Armour Laboratories. Study of the products was aided by the John and Mary R. Markle Foundation, the Douglas Smith Foundation for Medical Research of the University of Chicago, and the National Livestock and Meat Board.

Rats eating the low protein, low caloric basal diet gradually lose weight and become both anemic and hypoproteinemic, whereas those kept on the casein control diet gain weight and have normal serum protein and hemoglobin values. When the animals undergoing protein depletion have lost about one-third of their initial weight, a preliminary serum protein determination is made on each rat in the following way: Each animal is lightly anesthesized by ether, the tail is dipped into water for a few moments at a temperature of 45 to 50° C., and after having been dried and coated with a thin layer of vaseline, a vein is incised with a sharp lancet. The freely-flowing blood is collected in a small specially-made flanged vial which holds 0.5 cc. The bleeding is stopped by applying a small bandage of cotton and collodion. After the blood has clotted and the clot detached from the walls of the vial, the vial is tightly stoppered. Next morning, the retracted clot is withdrawn and, after centrifugation, protein determinations are made on the clear serum with a LaMotte densiometer, according to the method of Barbour and Hamilton. These determinations are always made between 12 and 24 hours after bleeding. The densiometer method is checked frequently with micro Kjeldahl analyses, utilizing larger quantities of serum; the results have checked consistently within 0.1 to 0.2 grams per cent over the range of 4.0 to 7.0 grams per cent. No attempt has been made to measure blood volume in view of the fact that Weech and his associates found a direct relationship between increase in concentration of serum albumin and increase in blood volume.

The values for total serum protein have varied as follows: For rats on the casein control diet, the levels have usually ranged between 6.5 and 7.0 grams per cent whereas those from rats kept on the basal ration have been between 3.5 and 5.50 grams per cent. The latter animals, which have values higher than 5.25 grams per cent, are eliminated from the current experiment and kept on the low-protein diet for use later. For the assays recorded in the present report, only protein-depleted rats whose serum protein values ranged between 4.0 and 5.25 grams per cent were utilized.

The rats selected for the repletion experiments, together with a few from the casein control diet group, are next placed in small individual cages and kept on their respective diets for about a week. Each rat in this selected group is weighed and, under ether anesthesia, 0.5 cc. of blood is removed from the heart by means of a 1 cc. syringe with a No. 24 gauge needle and the serum protein values are determined from this blood, using the densiometer method. After the serum protein determinations, the animals are immediately regrouped, on the basis of these serum protein values, for the repletion experiment. Usually from 4 to 6 animals are selected for each diet group and the groups are adjusted so that each contains animals comparable as to levels of serum protein, hemoglobin, and weight. Protein-depleted rats with serum proteins outside the 4.0 to 5.25 range are usually included in the group maintained on the low-protein diets as controls for the period of repletion.

Next morning the animals are weighed individually and are started on their respective repletion diets, supplied daily to each rat in 20-gram portions. The proteins to be assayed are mixed into the basal ration so as to make a final concentration, by weight (including the protein in the basal ration), of approximately 9 per cent (N × 6.25). The protein is substituted for an equal weight of corn starch. If the food being added also contains fat, carbohydrate, roughage, and moisture, these constituents are also substituted so that their final concentration in the test-diet will be the same as in diets 2E or 3E; in other words, the repletion diets are all approximately isocaloric, both with one another and with the basal and casein control rations. No attempt has been made to balance the diets so far as vitamins and minerals are concerned inasmuch as we assume that these accessory materials are already being fed in sufficient amounts and that added quantities would probably have no additive effect. Duplicate samples of each test-ration are analyzed for nitrogen content by the Kjeldahl method. At the end of each day during the repletion period, the amount of ration not eaten, if any, is weighed and the total amount eaten per rat per 24 hours recorded. With the exception of some of the incomplete proteins, the entire ration is usually consumed. During the repletion period, the rats are weighed on alternate days before being given their ration in order to determine gains or losses of weight. On the morning of the seventh day, the rats are weighed before feeding, and later in the day are again bled from the heart (0.5 cc.). The weights, and the values for serum proteins determined from this blood, are compared with the initial values, to establish gains or losses in one week.

In the present communication, only the results of the 7-day repletion are recorded. The data on the animals fed the casein control ration are not recorded inasmuch as they offer no information relevant to the present discussion.

The following experiments (I and II) present the detailed findings for the assay of the several proteins tested. Seven test rations containing the different proteins have been used as follows:

Ration 1 (2E and 3E). These two rations are the basal low protein mixtures described above. They differ only in that 2E contains no liver concentrate and only the amount of choline occurring normally in the dietary constituents. This ration was used in experiment I; ration 3E was used in experiment II. It should be noted, however, that, as judged by their inability to cause regeneration of serum protein, both were identical.

Ration 2—Crystallized bovine serum albumin. This highly purified serum albumin was prepared at the Armour Laboratories by the method of Cohn and Hughes, and in collaboration with the Department of Physical Chemistry, Harvard Medical School.

Ration 3—Bovine serum gamma globulin (Fraction II). This fraction of serum globulin, another product of the plasma fractionation method (5) carried out on bovine plasma at the Armour Laboratories, when tested electrophoretically, assays slightly better than 90 per cent gamma globulin. This is of especial interest in view of the fact that usually it is the gamma globulin fraction of immune serum which contains most of the antibody.

Ration 4—Dehydrated beef. This material was prepared by Wilson and Company, Chicago, Illinois, as Batch 142. Its composition is as follows:

Moisture	7.6 per cent
Protein	62.5 per cent
Fat	27.6 per cent
Ash	2.3 per cent

Ration 5—Gelatin. Gelatin was tested because of its possible value both as a blood substitute and for parenteral alimentation. This sample was supplied by Wilson and Company, Chicago, Illinois, as No. B 5162. It is derived from third run pigskin and has a nitrogen concentration of 15.9 per cent.

Ration 6—Isinglass. This was supplied by Dr. N. B. Taylor of the University of Toronto. It is a fine powder which contained a total nitrogen value of 16.3 per cent.

Ration 7—Corn germ, defatted. This material was prepared by the VioBin Corporation of Monticello, Illinois, as Sample No. 3697, Lab. No. A 775, and was used for comparison with the proteins of animal origin. Its composition was:

TABLE I

The influence of different proteins upon the regeneration of serum proteins and the regaining of weight in the hypoproteinemic rat

Rat No.	Initial values		Dietary protein and percentage in diet	Grams of protein consumed in 7 days	Body weight Gain or loss in 7 days		Serum proteins Gain or loss in 7 days	
	Serum proteins	Body weight				Per gram of protein consumed		Per gram of protein consumed
	grams per cent	*grams*			*grams*		*grams per cent*	
45-6	4.83	194	Low basal 2 E, 1.78 per cent protein	2.38	+1		+0.14	
42-5	4.94	208		2.20	−1		+0.03	
45-1	4.48	162		2.08	±0		−0.20	
Av.	4.75	188		2.22	0		−0.01	
41-4	4.97	186	Crystallized bovine serum alb., 7.82 per cent	10.40	+12	+1.15	+1.33	+0.13
47-2	4.41	172		10.47	+19	+1.82	+1.01	+0.10
43-4	5.25	229		10.94	+22	+2.05	+0.98	+0.09
50-3	4.90	134		8.91	+19	+2.13	+0.74	+0.08
46-7	4.65	201		9.85	+17	+1.73	+0.42	+0.04
Av.	4.84	184		10.11	+18	+1.78	+0.90	+0.08
47-1	4.38	208	Bovine serum gamma globulin, 8.72 per cent	11.59	+18	+1.55	+1.88	+0.16
45-3	4.86	201		11.51	+25	+2.17	+1.61	+0.14
41-3	5.00	182		11.07	+24	+2.17	+1.50	+0.14
41-5	5.21	203		11.94	+32	+2.68	+1.29	+0.11
50-4	4.65	149		10.72	+26	+2.43	+1.23	+0.12
Av.	4.80	189		11.37	+25	+2.20	+1.50	+0.13
47-3	4.41	181	Dehydrated beef, 9.04 per cent	12.65	+23	+1.83	+1.33	+0.11
46-2	4.86	178		12.65	+42	+3.32	+1.30	+0.10
42-2	4.69	201		12.65	+39	+3.08	+1.11	+0.09
47-4	5.11	200		12.65	+28	+2.21	+0.66	+0.05
Av.	4.77	190		12.65	+33	+2.61	+1.10	+0.09
45-2	4.80	191	Gelatin, 8.76 per cent	9.20	−3		+0.48	+0.05
45-5	4.90	165		9.98	−1		+0.35	+0.04
47-5	4.45	167		10.07	+3		+0.28	+0.03
Av.	4.72	174		9.75	±0		+0.34	+0.04

Protein	20.3 per cent
Fat	1.1 per cent
Fibre	3.7 per cent
Moisture	10.3 per cent
Ash	8.1 per cent
NFE	56.5 per cent

EXPERIMENTAL RESULTS

The results of experiments I and II are recorded in Tables I and II. Table I demonstrates the rapid regeneration of serum proteins in hypoproteinemic rats, fed crystallized bovine serum albumin, bovine serum gamma globulin, and dehydrated beef. All 3 also engendered a rapid regaining of weight, although the crystallized bovine serum albumin was somewhat less effective in both respects than bovine serum gamma globulin. The latter and the dehydrated beef

TABLE II

The influence of different proteins upon the regeneration of serum proteins and the regaining of weight in the hypoproteinemic rat

Rat No.	Initial values: Serum proteins	Initial values: Body weight	Dietary protein and percentage in diet	Grams of protein consumed in 7 days	Body weight Gain or loss in 7 days	Body weight: Per gram of protein consumed	Serum proteins Gain or loss in 7 days	Serum proteins: Per gram of protein consumed
	grams per cent	*grams*			*grams*		*grams per cent*	
62-4	4.10	103	Low basal 3 E, 2.14 per cent protein	2.82	−14		+0.31	
68-6	3.72	125		2.68	−27		+0.28	
66-4	4.69	133		2.40	+5		+0.21	
62-5	3.96	98		2.16	−8		−0.15	
67-6	3.90	125		2.16	−4		−0.15	
61-5	5.28	125		2.16	+11		−0.31	
Av.	4.28	118		2.40	−6		+0.03	
63-4	4.59	133	Crystallized bovine serum alb., 9.40 per cent	11.67	+21	+1.80	+1.29	+0.11
61-2	4.45	141		12.69	+22	+1.74	+1.11	+0.09
61-1	4.28	106		9.69	+16	+1.65	+0.97	+0.10
68-3	4.31	126		10.43	+14	+1.34	+0.90	+0.09
65-1	4.69	121		12.60	+26	+2.06	+0.42	+0.03
66-6	5.11	136		10.62	+15	+1.41	+0.35	+0.03
Av.	4.57	127		11.28	+19	+1.67	+0.84	+0.08
64-3	4.20	125	Bovine serum gamma globulin, 8.71 per cent	10.37	+24	+2.32	+2.00	+0.19
64-4	4.73	127		11.05	+23	+2.08	+1.88	+0.17
68-1	4.31	121		10.62	+27	+2.54	+1.70	+0.16
62-1	4.59	127		10.71	+14	+1.31	+1.61	+0.15
65-5	4.59	104		10.18	+30	+2.95	+1.50	+0.15
Av.	4.48	121		10.59	+24	+2.24	+1.74	+0.16
63-6	4.34	117	Dehydrated beef, 8.85 per cent	12.12	+35	+2.89	+2.02	+0.17
67-3	4.31	124		12.60	+36	+2.86	+1.90	+0.14
61-3	4.14	113		11.50	+35	+3.04	+1.95	+0.17
65-4	4.65	158		12.60	+31	+2.46	+1.92	+0.15
66-1	4.59	134		12.30	+38	+3.08	+1.74	+0.14
Av.	4.41	129		12.22	+35	+2.87	+1.92	+0.15
68-5	4.14	138	Isinglass, 8.69 per cent	9.13	−4		+0.20	+0.02
67-4	4.38	109		7.73	−3		+0.17	+0.02
64-2	4.04	163		6.17	−26		+0.06	+0.01
66-3	5.14	151		8.60	−6		−0.10	
Av.	4.43	140		7.91	−10		+0.08	
62-2	4.06	108	Corn germ, 8.34 per cent	10.33	+29	+2.80	+1.58	+0.15
67-5	4.16	124		11.34	+34	+3.00	+1.54	+0.14
65-3	4.24	149		11.68	+39	+3.34	+1.43	+0.12
66-2	5.18	177		11.68	+38	+3.26	+0.66	+0.06
Av.	4.41	140		11.26	+35	+3.10	+1.30	+0.12

were more nearly equal. Whereas greater increases in serum protein concentration resulted from the ingestion of gamma globulin, gains of weight were larger in the beef-fed animals. In contrast with these 3 proteins, gelatin was relatively ineffective in that the levels of the blood proteins were raised only slightly, and body weight was unaffected.

Table II records observations which, in general, confirm those of Table I. Here, too, crystallized bovine serum albumin was inferior to bovine serum gamma globulin and dehydrated beef. The dehydrated beef, moreover, produced greater gains in serum protein concentration than it did in experiment I. Nevertheless, the gamma globulin equaled dehydrated beef if measured by the grams per cent of serum protein gained per gram of protein consumed. This relationship, indeed, between the amount of protein consumed and the increase in concentration of serum proteins, would seem to be of particular significance.

As with gelatin in experiment I, isinglass was also an inadequate protein in that all 4 rats lost weight and their serum protein concentrations remained essentially unaltered. In fact, the influence of isinglass was not different from that of basal diet (3E) alone. In sharp contrast to the findings for isinglass were those for the corn germ protein. It was not quite the equal of dehydrated beef in producing serum protein but was as effective in increasing body weight.

A few fractions from human plasma have also been tested but, due to the small amounts of materials available, their effects were ascertained for a period of only 5 days, and in only a few rats. Nevertheless, the results indicate that the fibrinogen fraction was the best protein, nutritionally, followed by the gamma globulin, and a mixture of alpha and beta globulin.

COMMENT

The differences in results of experiments I and II require brief comment because of variations in conditions. The basal ration in experiment II contained additional choline, as well as liver concentrate. Furthermore, the rats in this experiment were younger when the depletion diet was begun and were more hypoproteinemic and uniform in size at the start of the repletion period. Because of the lack of uniformity among the rats in these 2 experiments, it is obvious that during the period of repletion, some variability is inevitable. This is to be expected, particularly when proteins of closely similar biological potencies are tested in only a few animals. Averages, therefore, should be interpreted merely as suggesting trends rather than affording statistically valid estimates of biological quality.

There is still uncertainty concerning the degree of protein depletion necessary in an animal in order to demonstrate most effectively the abilities of different foods to cause the regeneration of serum proteins. Whipple and his associates (4) assume that, in general, the greater the degree of hypoproteinemia, the greater is the stimulus for protein regeneration. It is for this reason that they maintain their hypoproteinemic dogs at a plasma protein level of approximately 4.0 grams per cent. An added advantage of a marked hypoproteinemia is that it affords a wider range in which to measure variations in protein potencies. Furthermore, a more marked reduction of the protein reserves may serve to reveal the incompleteness of a test protein which might otherwise be masked by the complementing action of protein materials available in the reserve stores. For these reasons, therefore, we have subjected our rats to prolonged protein depletion (60 to 94 days) before using them for a dietary assay.

Despite this long period of protein depletion, we have seen no evidence of injury to the mechanism which produces the serum proteins. Thus far, we have assayed some 21 different proteins of animal and vegetable origin in more than 200 hypoproteinemic rats. In general, the proteins which, by other standards, are usually rated as proteins of good biological quality have caused a rapid regaining of weight and a quick increase in the concentration of serum proteins. In contrast, proteins of poor biological quality have yielded poor results. For example, 7 rats fed dehydrated beef in previous experiments have shown an average increase in percentage concentration of serum protein of around 2.0 grams, whereas 9 rats fed gelatin have shown an average increase of only 0.06 gram. It is possible, therefore, by this type of assay, to establish a rating of biological potencies for many varieties of proteins.

The theoretical implications of these experiments are of particular interest with respect to their demonstration of the superiority of gamma globulin as an effective stimulus in a starving animal, both for the regeneration of serum protein and for the rapid recovery of weight loss. We conclude that this means that beef serum gamma globulin contains a large assortment of readily available essential amino acids. Furthermore, in view of the fact that most antibodies are gamma globulins, these findings suggest that the process of antibody production must also require an ample supply of essential amino acids, either in the diet or in the protein reserves, in order to permit the fabrication of antibodies in normal quantities and at a normal rate (6). Without these amino acids, it is unlikely that a hypoproteinemic animal can regain its ability to synthesize antibody-globulin when fed only incomplete proteins. Experiments now in progress in this laboratory tend to confirm this assumption.

SUMMARY

A method is described for the quick determination of the biologic quality of a protein when fed to the hypoproteinemic rat. The efficiency of the protein is evaluated by its capacity to increase the concentration of serum proteins and to produce a gain in weight within a 7-day period. Variations due to small differences in degrees of weight and of hypoproteinemia, as well as to natural differences between animals, are compensated for by the use of groups of rats. This method of assay permits a separation of biologically adequate and inadequate proteins and gives information about differences of quality. A protein is considered to be good when the concentration of serum proteins is significantly increased and is high in relation to the quantity of protein ingested.

According to these criteria, bovine serum gamma globulin is highly efficient, especially with respect to serum protein regeneration. In fact, it practically equals dehydrated beef, the best protein so far tested. Crystallized bovine serum albumin, although a fairly adequate protein, is less effective, and corn germ protein compares favorably with both dehydrated beef and bovine serum gamma globulin. In contrast, the incomplete proteins, gelatin and isinglass, seem to possess no distinct ability in either respect.

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THE IMMUNE RESPONSE OF HUMAN BEINGS TO BRIEF INFECTIONS WITH PNEUMOCOCCUS[1]

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As a result of qualitative and relative tests (1, 2), it is generally agreed that rapid termination of lobar pneumonia in man by means of drugs of the sulfanilamide group does not prevent development of the immune response long recognized as characteristic of spontaneous recovery from the disease and evidenced by the formation of type-specific anticarbohydrate. Nothing is known, however, of the magnitude of this immune response in absolute terms, beyond a single report of 0.15 mgm. of anticarbohydrate nitrogen per ml. in the serum of a patient during convalescence from lobar pneumonia due to Type I pneumococcus (3). This patient had received neither serum nor curative drug. The present series of measurements is therefore believed to be the first extended quantitative record of an immune response of human beings to a typical, relatively brief period of infection. Several cases treated with penicillin are included in the series. Since detailed clinical studies on similar case material, in which only qualitative, or relative, immunological methods were used, have already been published, as noted herein, emphasis will be given rather to the new methods used and the deductions made from the analytical data. It is hoped that these methods may be found applicable to other problems of clinical research.

A serious complicating factor was discovered in the course of this and parallel studies. One of the immunologically active components of pneumococci of all types, even of the unencapsulated forms which are devoid of type-specificity, is the somatic polysaccharide or "C"-substance (4). It was found, in an investigation to be published elsewhere, that while the sera of normal persons contained no more than traces, at most, of antibodies to the type-specific polysaccharides of Types I, II, III, IV, and V pneumococci, almost all contained measurable amounts of antibody to pneumococcus C-substance. Hence, before a reliable assay could be obtained of the type-specific antibody response in pneumonia patients, it was all the more necessary to remove anti-C in a preliminary absorption, since almost all samples of the type-specific carbohydrates contain C-substance as an impurity. Thus, variable quantities of anti-C would be precipitated with the type-specific antibody and gross errors might result. In the work now to be reported, this preliminary absorption was carried out quantitatively and the figures are included in the table.

CASES

Twenty-three adult patients, hospitalized for pneumococcal infections, were studied. One patient (III-2) was admitted with a physical examination and history typical of an acute meningitis following an acute otitis media. Pneumococcus Type III was cultured from the infected ear, but the spinal fluid cultures were consistently negative. The remaining patients had pneumonia; of these, 16 presented the classical picture of lobar pneumonia.

All patients received sulfathiazole, sulfadiazine, or penicillin therapy, and blood concentrations were maintained at adequate levels as long as appeared indicated. Data from the patients' charts are included in Table I.

ANALYSES FOR ANTIBODY CONTENT

The method used was that previously reported (5). Merthiolate[2] up to 1:10,000 was added to all sera and analyses were run under conditions favoring sterility, owing to the long period involved. In order to avoid the uncertainties due to human complement (6), this was first removed from the sera by admixture of triplicate 3 to 4.5 ml. portions with 1 ml. of a dilution of rabbit anti-egg albumin serum containing about 0.4 mgm. of antibody nitrogen and 1 ml. of a 0.9 per cent saline solution of crystalline egg albumin (Ea) containing about 0.04 mgm. of Ea N. This was found preferable to heat inactivation, which destroyed a portion of the antibody in sera of low

[1] The work reported in this communication was carried out under the Harkness Research Fund of the Presbyterian Hospital, New York.

[2] Manufactured by Eli Lilly and Co., Indianapolis.

TABLE I

Clinical summary and precipitin response of patients with pneumococcal infections

Pneumococcus type, case number	Age of patient	Lobes involved	Blood culture	Approx. day of disease drug started	First whole day temp. <100°	Further days to serum sample analyzed	Antibody to pneumococcus "C" substance	Homologous anticarbohydrate	Pneumococcus antiprotein (total N)
	years						*mgm. N per 4 ml. serum*		
LOBAR PNEUMONIA									
I—3	34	1	−			*ca.* 120	0.009	0.001	
I—5*	64	1	−	>4	22	8	0.027	0.017	
I—6	38	1	−	3	8	8	0.018†	0.025†	
II—2‡	36	1	−	3	7	3	0.036	0.498‖	0.027
II—3‡	38	1	−	2		9	0.023	0.026	0.002
III—5‡	43	1	−	2	9	34	0.010	0.001	
III—7**	49	1	−	2	6	10	0.032	0.012	
V—2	70	1	+	2	9	3	0.051	0.286	0.003
VI—1	22	1	−	1	1	6	0.143	0	0.017
VII—7††	56	1	−	4	5	11	0.045	0.006	
VII—8	63	1	+	4	6, 14	10	0.013	0.062	
VII—10‡	37	1	−	3		7	0.053	0.067	
VII—11	49	1	−	4	9	7	0.051	0.138	0.007
XIII—1	56	2	−	3	11	12	0.114	0.003	0.010
XIV—1	28	1	−	3	8	8	0.078	0.019	
XIX—1	52	1	−	4	6, 8	7	0.019	0.016	
BRONCHIAL PNEUMONIA									
III—4	33		−	3	5	4	0.026	0.009	
III—6	51		−	2	9	7	0.045	0.005	0.003
VII—6	60		−	4	11	12	0.004	0.123	
XVII—1	57			14	17	17	0.030	0.002	
XIX—2	45			4	8	4	0.097	0.064	
XXIX—1	71		−	7	8	9	0.036	0.003	
MENINGITIS									
III—2	53		−	2	5	53	0.016	0.277‡‡	

Patients were treated with sulfadiazine unless otherwise noted.
* Received penicillin and then sulfadiazine. Agglutination titer of serum, 1 : 2.
† Independent analyses on another portion of the same serum sample again gave 0.018, 0.025 mgm. N for anti-C and anti-I, respectively.
‡ Treated with penicillin.
‖ Antibody N by micro-Kjeldahl method, 0.510 mgm. Agglutination titer, 1 : 64 to 128.
** Infection resistant to sulfadiazine, treated with penicillin.
†† No drug-treatment.
‡‡ Micro-Kjeldahl. Antibody N per 4 ml., without prior removal of anti-C, 0.333 mgm. A serum sample taken 32 days earlier, from which the anti-C was not first removed, showed 0.37 mgm. antibody N per 4 ml.
A rabbit antipneumococcus Type I serum pool, diluted to an antibody content of 0.5 mgm. N per 4 ml., showed an agglutination titer of 1 : 40 under the conditions used in estimating the 2 titers given above. Titers and antibody contents were roughly parallel in the 3 instances.

content but was permissible with the stronger sera. The Ea-anti-Ea precipitations were carried out at room temperature and allowed to stand until the precipitates flocked (7), after which the tubes were left in the ice-box for 48 hours and centrifuged in the cold.[3]

Aliquot portions of the supernatants were set up as follows in sterile, tapered, Pyrex centrifuge tubes of about 7.5 ml. capacity. Three portions were measured out, one serving as a blank. To the other two, 0.01 or 0.02 mgm. of pneumococcus C-substance was added in a volume of 0.1 or 0.2 ml. It was found essential to use C-substance derived from a type of pneumococcus other than that causing the infection in the patient whose serum was being analyzed, for it is as difficult to remove the last traces of type-specific substance from a sample of C-carbohydrate as to eliminate the last residues of C-substance from samples of type-specific carbohydrate. Alternatively, C-substance, derived from wholly degraded pneumococcus R (Dawson S) strains free from type-specific carbohydrate, may be used to advantage.

The contents of the tubes were thoroughly mixed with a

[3] In an International Equipment Co. refrigerated centrifuge.

thin sterile glass rod or by rapid twirling. After capping with sterile rubber caps the tubes were placed in a water-bath at 37° C. for one-half to one hour and were then allowed to stand 8 days in the ice-box, with occasional twirling. In the strongest sera, precipitates often formed overnight but most of the weaker sera remained clear and required several days before the first small floccules separated. The tubes were finally centrifuged in the cold. The supernatants of tubes in which relatively large precipitates had formed were allowed to stand another 8 days after mixing with an additional 0.01 or 0.02 mgm. of C-carbohydrate. The precipitates and blanks were washed in the cold in the usual way (8) with chilled saline, stirring well, or twirling vigorously to ensure maximum disintegration of the precipitate and thorough mixing. When a stirring rod was used, it was rinsed down with a little chilled saline. Supernatants from the washings were often recentrifuged in order to recover any precipitate carried over, usually combining the washings from duplicates in larger centrifuge tubes. A third washing was usually given the tubes in which the washings were recentrifuged. Precipitates were dissolved in a few drops of 0.1 N sodium hydroxide, using a hand lens if necessary to make sure that the sometimes glassy precipitate was completely dissolved. The solution of the traces of precipitate recovered on recentrifugation was divided approximately equally among the 2 duplicates. The volumes were then made up with water to a calibration mark (usually 2.5 ml.) in the tubes, or the solutions were transferred to 5.0 or 10.0 ml. measuring flasks when the precipitates were relatively heavy. Two ml. portions of the duplicate solutions of dissolved precipitate were analyzed for nitrogen (5).[4] One hundredth to 0.04 mgm. of protein or antibody nitrogen in the aliquot proved most satisfactory, and in this range, successive determinations usually varied by only a few thousandths of a mgm. The tyrosine color value of the antibody was checked by direct comparison of the colorimetric with the micro-Kjeldahl method.

The supernatant sera from the anti-C analyses, including the blank, were carefully drained into sterile centrifuge tubes, such as those suggested above. To the duplicates from the anti-C estimations were then added amounts of the homologous type-specific carbohydrate,[5] depending upon the result of a preliminary test with 1 ml. of the patient's serum. Usually, 0.005 to 0.05 mgm. sufficed. The analyses were carried out in the same manner as those for anti-C[6] and supernatants were treated with more specific polysaccharide if the precipitates were relatively large. In occasional sera, such as II-2 and III-2, precipitates formed almost as quickly as in hyperimmune rabbit sera and analyses could be carried out by the micro-Kjeldahl method (8), or as described above with as little as 1 ml. of serum.

After removal of anti-C and type-specific antibody, most of the sera from cases infected with pneumococci of types other than VII gave small precipitates with the specific polysaccharide of Type VII pneumococcus as well, but as the amounts rarely exceeded a few thousandths of a mgm. of nitrogen, the significance of these precipitates is doubtful. Possibly, they represented additional traces of anticarbohydrate which would have been included in the preceding analyses had they been allowed to stand even longer. However, the formation of these precipitates makes it appear less probable in the Type VII cases than in those of the other types that the entire type-specific antibody response was due to the immediately preceding infection.

A few sera were also analyzed for antibody to pneumococcus protein after the anti-carbohydrate analyses were completed and the data so obtained are included in the table. Finally, supernatants were tested with appropriate immune rabbit or horse sera to make sure that an excess of C, type-specific carbohydrate, or protein had been used in the analyses.

The procedure, while laborious and time-consuming, is especially well adapted to the analysis of large numbers of sera, as the work may readily be planned so that analyses on sets of 4 to 6 sera become due daily.

In order to determine whether the anti-C values represented true antibody to the somatic polysaccharide of pneumococcus, or consisted wholly or in part of "C-protein," the material reactive with C-substance in the albumin fraction of the serum of febrile cases (4, 10), the following experiment was carried out:

A pool was made of 3 sera which had averaged about 0.1 mgm. of anti-C per 4 ml., an unusually large amount. Twenty-four and a half ml. were diluted with 25 ml. of water and the globulin fraction, which would presumably contain true antibody to C-polysaccharide, was precipitated by addition of 49.5 ml. of warm saturated sodium sulfate solution. The precipitate (*A*) was centrifuged off as sharply as possible and a fraction (*B*) was precipitated from the supernatant by addition of 50 ml. of warm saturated sodium sulfate solution, as recommended (10b) for the separation of the albumin fraction containing any "C-protein" present. This was filtered off after standing overnight. Both *A* and *B* were dissolved in water with the aid of a drop of N sodium bicarbonate solution, di-

[4] In this preliminary note, the directions on p. 405 should read: "Aliquots of 2.0 ml. are mixed with 6 ml. of clear 12.5 per cent Na_2CO_3 solution and 1 ml. of 0.1 per cent $CuSO_4 \cdot 5H_2O$ solution and allowed. . . ."

[5] The specific polysaccharides of Types I, II, III, and VIII pneumococcus were prepared in this laboratory according to (9). The others were furnished through the courtesy of Drs. W. E. Bunney and J. Palmer of E. R. Squibb & Sons, New Brunswick, N. J., and Drs. A. B. Wadsworth and Rachel Brown of the New York State Dept. of Health Research Laboratories, Albany, New York.

[6] In a detailed analytical paper in preparation for publication, it is shown that known quantities of type-specific antibody from horse or rabbit antipneumococcus sera added to normal horse or rabbit serum (0.010 to 0.040 mgm. of antibody nitrogen per 4 ml. of serum) are recoverable to the extent of 80 to 90 per cent by the method.

alyzed in the cold against several changes of 0.9 per cent saline, made up to 1:10,000 with merthiolate, and finally centrifuged. The volume of *A* was 22 ml., that of *B*, 18 ml. Analyses for anti-C were carried out with the largest possible blank and duplicate portions, as described above, yielding, after several successive additions of C-substance, 0.123 mgm. anti-C nitrogen in the globulin fraction *A* and zero anti-C in the albumin fraction *B*, even after addition of 10 mgm. of calcium chloride to the latter in case absence of precipitation had been due to a lack of calcium ion.

DISCUSSION

As previously noted (6), the estimation of small quantities of antibody in human sera presents unexpected difficulties and these are added to in the study of antibodies to pneumococcus by the normal occurrence of antibodies to the somatic carbohydrate or C-substance of this microorganism. These antibodies differ from the anti-C present in febrile attacks due to many microorganisms (4, 10) in the time of their appearance, in their occurrence with other antibodies in the globulin fraction, and in that the specific precipitates which they form with C do not require the addition of calcium ion in order to keep them in an insoluble form. The reason for the presence of anti-C in normal humans remains uncertain, although it may be recalled that pneumococci are usual inhabitants of the human nose and throat. Possibly C-substance in antigenic combination with protein, as in meningococci and hemolytic streptococci (11), is a dominant antigen of these non-pathogenic forms anP makes its presence evident in varying degreei As noted in the table, the anti-C content of a patient's serum is often equal to or greater than that of the type-specific antibody elicited by the infection. That the latter actually arises from the infection seems evident from numerous analyses on normal sera carried out in this laboratory with a variety of type-specific polysaccharides. Such response as was indicated in a few instances was usually traceable to the C-substance in the type-specific carbohydrate used except that, in the case of the type VII polysaccharide, a small residuum of type-specific antibody was evident in a fair proportion of normal sera. In only one instance (VI-1) was a sample of serum available prior to the pneumococcus infection. In this case, the unusually high anti-C content was present before the attack of lobar pneumonia, as well as after, and failed to protect the patient from infection with Type VI pneumococcus.

With regard to the analytical technic, the use of type-specific pneumococcus suspensions did not appear suitable for the determination of antibodies in the sera of humans convalescent from pneumonia owing to the ordinarily small difference between the nitrogen in the washed pneumococcus suspension used for the analysis and the total nitrogen after addition of the antibody, a difference often of the same order of magnitude as the analytical errors in the method. When type-specific polysaccharides were used, instead, for precipitation of the antibody, it was not only found that deposition of specific precipitate was usually incomplete in the time ordinarily allotted (8), but also that the complement present in fresh human sera introduced further uncertainties. These have been more fully discussed elsewhere (6). Removal of the complement by heat inactivation seriously damaged the small amounts of anticarbohydrate present in the weaker convalescent sera, but elimination of complement was successfully accomplished by its fixation to an unrelated immune system such as egg albumin—anti-egg albumin. Analysis of the decomplemented sera could then be carried out with precautions to ensure sterility and by allowing the tubes to stand much longer than had been found necessary with the sera of hyperimmunized animals.

By the use of the phenol reagent (5), amounts of antibody, roughly one-tenth those ordinarily necessary for the micro-Kjeldahl modification hitherto used (8), may be estimated with equal accuracy and the scope and utility of the quantitative method is thereby greatly extended. As carried out in this way, the precipitin reaction not only yields quantitative data on antibody content in weight units, but is actually made far more sensitive than the complement fixation reaction in comparable immune systems. While the limiting amounts of antibody nitrogen are roughly of the order of 0.001 mgm. per ml. in both reactions (5, 12), in complement fixation tests this quantity of antibody must be present at a serum dilution of 1:5 or 1:10 in order to avoid non-specific or anticomplementary effects. With the new precipitin technic, however, these

minimal quantities of antibody nitrogen are accurately determinable in undiluted serum, so that sera too weak for complement fixation may be assayed.

As noted in the table, human sera resulting from pneumococcus infections abbreviated or terminated by the use of sulfa drugs or penicillin show an extremely varied immune response on the part of the patient, from complete absence of type-specific anticarbohydrate to a content of antibody almost equal to that reported in an untreated convalescent by Kabat (3). Indeed, the variation is not greater than might be expected in an equal group of rabbits or horses, the two species of animals ordinarily used for the production of antipneumococcus sera. When one considers the length of time necessary and the quantity of pneumococci employed for the production of potent antisera in rabbits and horses, the antibody response of this group of humans to what one might term a short course of immunization would seem just as favorable.[7]

The precise analytical data given above not only indicate the actual content of circulating anticarbohydrate in a representative group of drug-treated pneumococcus infections but also furnish an extension and confirmation of earlier studies on pneumonia patients in which mouse protection or agglutinin titers (2, 13 to 15) or qualitative precipitin tests (15a, 16) were evaluated. As in the earlier, relative studies, no correlation appears in the present series between the more obvious clinical factors and the antibody response. Since the wide variation in this response is probably as characteristic for humans as for other animals, it is apparent that a very large number of cases would be needed to establish any such correlation.

Investigation of antibodies to pneumococcus in human sera under various circumstances is being continued.

SUMMARY

1. Technical details are given for overcoming the difficulties and increasing the sensitivity of precipitin estimations in human sera so that the results may be used in accurate studies of the antibody response in infectious disease. Pneumococcus anticarbohydrate may now be estimated quantitatively at concentrations lower than detectable qualitatively by complement fixation.

2. Quantitative data, in milligrams of antibody nitrogen per unit of volume, are presented on the immune response of patients to pneumococcal pneumonia and meningitis terminated by sulfa-drug or penicillin therapy. Values are given for the antibodies precipitable by C-polysaccharide, type-specific polysaccharide, and nucleoprotein. The figures found varied from zero to as much as 0.5 mgm. of antibody nitrogen per 4 ml. of serum.

3. The type-specific anticarbohydrate response in the drug-treated patients was of a magnitude and variability comparable with that of rabbits or horses given a brief course of immunization.

The writers are indebted to numerous members of the clinical staff of the Presbyterian Hospital and to Professors Colin M. MacLeod and William S. Tillett of the New York University Medical School for the sera used, also to Miss Betty Robinson for a number of the analyses.

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THIOURACIL. ITS ABSORPTION, DISTRIBUTION, AND EXCRETION [1]

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In a previous paper (1), we have mentioned various studies dealing with thioureas and sulfonamides which have led to the use of one of the thiourea derivatives, thiouracil, in the treatment of patients with thyrotoxicosis (2, 3). Evidence continues to accumulate that when this drug is administered to patients or experimental animals, the production of thyroid hormone is inhibited, resulting in a fall of the basal oxygen consumption, increased activity of the anterior pituitary, and consequent hyperplasia of the thyroid gland. We have also pointed out (1) the importance of knowing more about the pharmacological properties of this drug, particularly the rate of absorption and excretion and its metabolism. For these studies, we found that it was necessary to establish methods for the estimation of this substance in tissues and body fluids. The methods which we found suitable (1) are based on Grote's observation (4) that a color develops when substances of a C=S type are treated with a special sodium nitroferricyanide reagent. Thiouracil [3] has the C=S linkage and we found that it gives a greenish color which can be estimated by means of an Evelyn photoelectric colorimeter. We refer the reader to our previous report (1) for a consideration of the specificity of the method. However, we wish to state here that whereas we have not proved directly that all of the color obtained is attributable to thiouracil, the evidence strongly suggests that such is the case.

ABSORPTION AND EXCRETION

In conducting the experiments relating to the absorption and excretion of thiouracil the following points were investigated:

(*1*) The rate of disappearance of the drug from the gastro-intestinal tract.

(*2*) The quantity broken down in the stomach and intestines, either by enzymes or by bacteria.

(*3*) The proportion of thiouracil excreted as such in the stools.

(*4*) The interval between its ingestion and its appearance and accumulation in the blood and urine.

(*5*) The frequency of administration and the total dosage necessary to establish and maintain a constant level in the blood stream and a constant rate of excretion in the urine.

(*6*) The interval between the discontinuation of treatment with the drug and its disappearance from the excreta.

(*7*) The proportion of the total amount of thiouracil ingested which is recoverable from the urine and feces.

A few of the studies were found to be conveniently performed with rats, but most of them were carried out in man.

Experiments with rats

The rats used were of the Wistar strain. They weighed about 200 grams and were not fasted.

The purpose of the first experiment was to determine the rate of absorption of thiouracil from the gastro-intestinal tract and the segments from which it is absorbed. Each of 3 rats was given 5 cc. of a 20 mgm. per cent solution of thiouracil by means of a stomach tube. Two hours later the animal was killed by a blow on the head and sutures were placed in such a manner as to isolate the stomach, duodenum, jejunum, and ileum. These segments were ground and their content of thiouracil was determined (Table I). A small portion of the drug was found in various segments, but from 76 to 91 per cent of the amount ingested had disappeared.

In another experiment, using ether anesthesia,

[1] This investigation was aided by a grant from the Milton Fund of Harvard University.

[2] Fellow of the Commonwealth Fund.

[3] The thiouracil was supplied by the Lederle Laboratories, Inc., Pearl River, New York.

TABLE I

Disappearance of thiouracil from the gastro-intestinal tract

Percentage of total drug ingested remaining after 2 hours			
Site	Rat 1	Rat 2	Rat 3
Stomach	16	0	5
Duodenum	3	0	0
Jejunum	0	9	0
Ileum	5	2	4

the proximal and distal lumina of the stomach, duodenum, and jejunum of 4 rats were occluded with sutures. Care was taken to avoid unnecessary trauma. Into each isolated segment was injected 1 cc. of a 20 mgm. per cent solution of thiouracil. A very small needle was used for injecting and the site of injection was immediately cauterized with a hot rod. At intervals of 1, 2, and 3 hours later, the animals were killed by means of a blow on the head and the amount of thiouracil remaining in the segments was determined (Table II). The drug was found to disappear rapidly from the stomach, duodenum, and the jejunum, in most instances more than 80 per cent having disappeared. In one animal, none of the drug remained in any segment at the end of 2 hours. However, this does not necessarily mean that all of the thiouracil had been absorbed; some of it may have been broken down by gastro-intestinal enzymes or by bacteria. Consequently, we investigated these possibilities by incubating a known amount of thiouracil with the contents of the gastro-intestinal tract. Four non-fasted rats were anesthetized with ether and, by means of sutures, the stomach, duodenum, jejunum, and ileum were isolated. Immediately thereafter, 0.5 mgm. of pilocarpine hydrochloride was injected subcutaneously. At intervals of 1, 2, and 2.5 hours, the animals were killed and the contents of the isolated segments were removed. To the contents was added enough of a 20 mgm. per cent solution of thiouracil to make a final concentration of from 4 to 10 mgm. per cent. The mixture was incubated at 37° C., the interval being 12 hours in the case of 2 animals and 2 hours in the case of the other 2. The stomach contents produced an average destruction of 42 per cent of the added thiouracil (Table III). The duodenal, as well as the jejunal contents, caused an even greater destruction, whereas the ileal material had no effect in any experiment. Therefore, the disappearance of the thiouracil from the isolated segments of the gastro-intestinal tract may have been partially due to destruction of the drug, rather than being due entirely to absorption.

Further studies were conducted to determine more definitely the amount of drug absorbed from the gastro-intestinal tract and the amount destroyed therein. Eleven rats were given 5 cc. of 100 mgm. per cent thiouracil through a stomach tube and one hour later they were killed by a blow on the head. Immediately thereafter, the gastro-intestinal tracts of 7 animals were removed, without spilling any of the contents, and the total amount of thiouracil was determined in 4 cases. The carcasses of these 7 animals, as well as those of the 4 rats with the gastro-intestinal tract intact, were homogenized by means of a sausage grinder and a Waring blendor and the total amount of thiouracil was determined. All urine passed during the hour was added to the carcass mixture. In analyzing the results, we have considered the amount of the drug unaccounted for as being the maximal amount destroyed in the gastro-intestinal tract. However, the actual quantity destroyed is, no doubt, somewhat less than this since the recovery

TABLE II

Rate of disappearance of thiouracil injected into isolated segments of gastro-intestinal tract

Hours	1	2	2	3
	Percentage of disappearance			
Stomach	85	100	10	85
Duodenum	85	100	40	96
Jejunum	38	100	100	99

TABLE III

Rate of destruction of thiouracil incubated with the contents of segments of the gastro-intestinal tract

Hours	1	2	2.5	2.5
	Percentage of destruction			
Stomach	41	43	45	40
Duodenum	68	78	70	51
Jejunum	75	0	88	41
Ileum	0	0	0	0

of the drug is probably not quite 100 per cent and since some destruction may have taken place in the tissues (see below). Nevertheless, there is no question that the least amount of the drug absorbed was the quantity found in the carcasses without the gastro-intestinal tract. This quantity, although variable, tended to be about 50 per cent of the amount administered (Figure 1). The amount destroyed was in most instances less than 25 per cent.

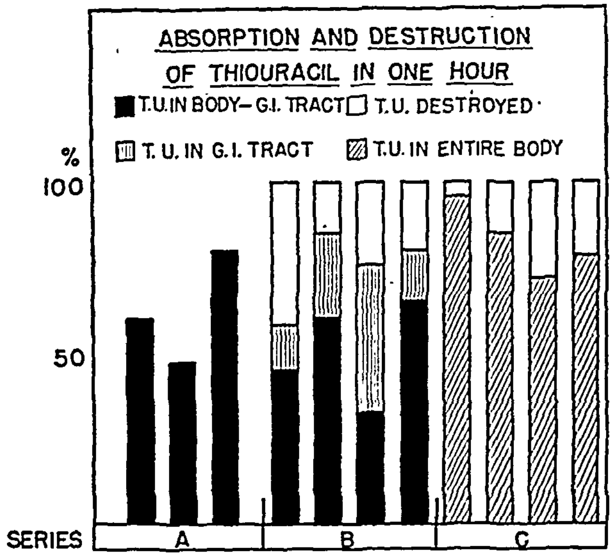

FIG. 1. ABSORPTION AND DESTRUCTION OF THIOURACIL IN ONE HOUR

Each column represents in one rat the percentage of the total amount (5 mgm.) of thiouracil (T.U.) given intragastrically, which was recovered from the body and the gastro-intestinal (G.I.) tract one hour later. In series A was determined the thiouracil content of the body without the gastro-intestinal tract. In series B, the gastro-intestinal tract was also examined, while in series C the entire animal was analyzed as one specimen. Note that the absorption of the drug by the body tends to be greater than 50 per cent and the destruction is less than 25 per cent. These must be minimal and maximal averages, respectively, for reasons discussed in the text.

Clinical observations

The amount of thiouracil remaining in the gastro-intestinal tract at autopsy was studied in 3 cases. At the time that the drug treatment was instituted, death was imminent in each case. One patient had nephritis and was given only one dose, 1 gram administered 10 hours before death. Each of the other patients had a cerebral hemorrhage and was given 0.2 gram at intervals of 4 hours for 2 to 3 days. The last dose in each case was administered about 4 hours before death. At autopsy, a small amount of the drug, less than 25 mgm., was found in the stomach and duodenum of each case but none was found in the jejunum. These findings indicate that absorption is rapid in spite of a moribund state and that possibly all of the drug is absorbed (or destroyed) before it reaches the jejunum.

A normal man, aged 32, was given 0.2 gram of thiouracil by mouth. At the time of the medication, he was in a fasting state and was not permitted to eat until 2 hours later. Blood specimens were obtained, following the ingestion of the drug, at 15 and 30 minutes, hourly intervals for 4 hours, 2-hour intervals for 8 hours, and at 1, 2, and 3 days. All urine was saved and collected at about the same time as the blood specimens. The highest blood level, 2.3 mgm. per 100 cc., was attained in the 15-minute specimen (Figure 2). Thereafter, it began to fall, reaching a relatively low value within 8 hours, 0.3 mgm. per 100 cc., remaining at about this level until the end of the third day when no more of the drug was found. A small amount of thiouracil was found in the 30-minute specimen of urine. The maximal excretion, 15 mgm., occurred during the second hour; thereafter the excretion progressively decreased, less than 3 mgm. being excreted during the second and third days. Throughout the period of observation, only 94 mgm., or 47 per cent of the dose administered, was excreted in the urine.

The normal subject, mentioned above, was used in another study 3 weeks later. He was given 0.2 gram of thiouracil every 4 hours for 3 days. Blood and urine samples were obtained at frequent intervals throughout a period of 7 days, as illustrated in Figure 3. After 12 hours, the blood level remained relatively stationary, 2.5 to 3 mgm. per 100 cc., but it showed a marked drop 16 hours after discontinuing the drug. Essentially all of the drug had disappeared from the blood stream within 48 hours after cessation of treatment. A maximum rate of excretion of the drug in the urine occurred during the 4- to 8-hour interval, 75 mgm. being excreted. Four hours after discontinuing the use of thiouracil, the rate of its excretion began to decline rapidly;

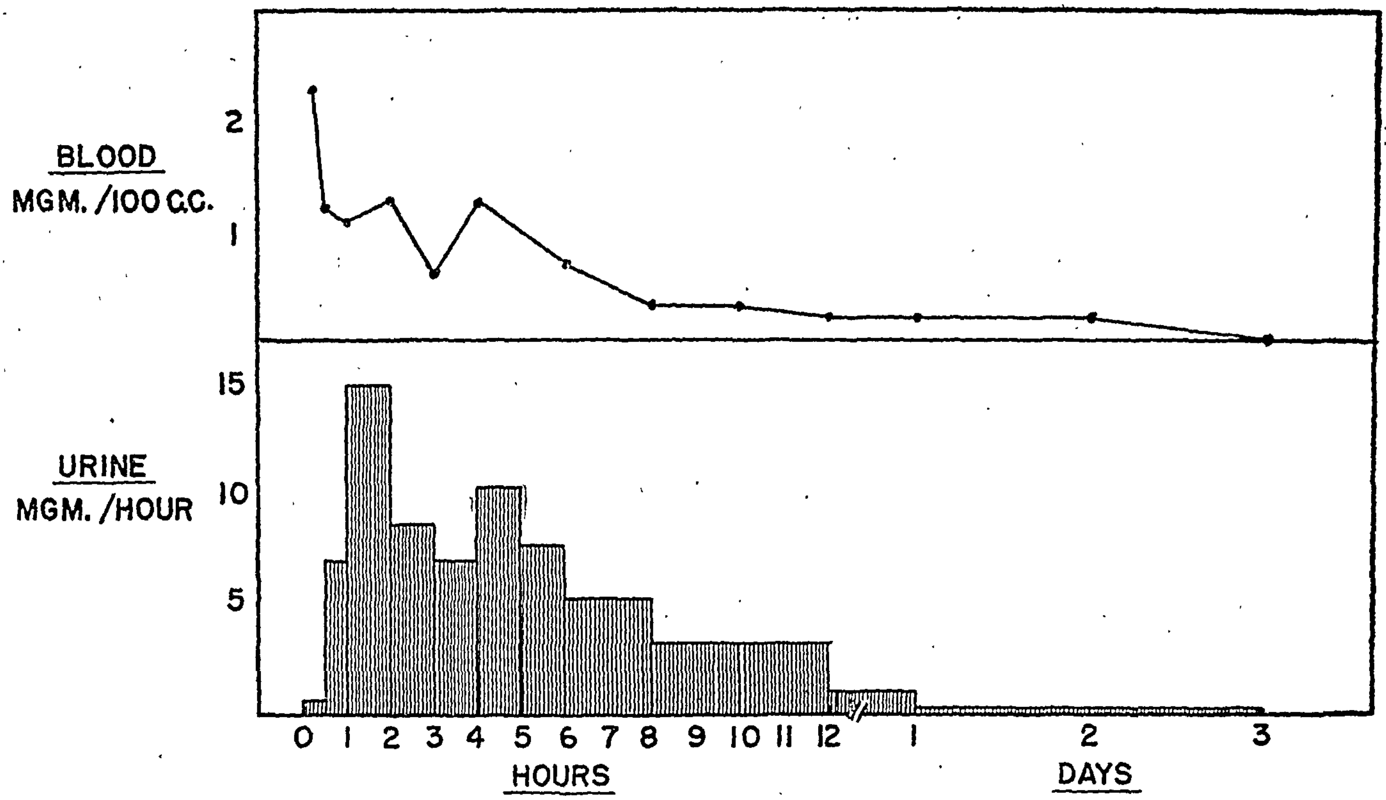

FIG. 2. THE AMOUNTS OF THIOURACIL IN THE BLOOD AND URINE OF A NORMAL INDIVIDUAL FOLLOWING THE INGESTION OF 0.2 GRAM OF THE DRUG

however, it did not disappear from the urine until 4 days later. Throughout the period of observation, only 1.3 grams, or 34 per cent of the total amount of thiouracil ingested, was excreted in the urine.

A man aged 42, who was normal except for marked obesity and mild diabetes, was given 0.2 gram of thiouracil every 4 hours. Blood and urine specimens were obtained at hourly intervals for 8 hours, at 2-hour intervals for 4 hours, and daily thereafter for 6 days. Thirty minutes after the first dose the blood was found to contain 2.3 mgm. per 100 cc. (Figure 4), but within another 30 minutes, it had fallen to 1.2 mgm. per 100 cc., remaining at this approximate level until the next dose was given. One hour after the second dose, the concentration was 3 mgm. per 100 cc., but thereafter continued to fall until the third dose was administered. However, after the first 24 hours, the level was found to remain relatively constant during the 5 days that it was followed. Small amounts of thiouracil were excreted in urine during the 20 minutes following the ingestion of the first dose of the drug, and there tended to be a progressive increase in the rate of excretion until the end of the first 24 hours. For the next 5 days, the rate of excretion remained strikingly constant, being about 510 mgm. per day.

In Figure 3 are plotted the concentrations of thiouracil in the whole blood of 8 individuals. Subject 5 was a normal man, subject 8 had mildly active rheumatic fever, subject 3 had mild diabetes, and the other 5 had thyrotoxicosis. Some variability in the rate of elevation of the blood level is observed; however, a relatively constant level was reached in most instances within 24 to 48 hours. The responses to the smaller doses were not very different from the responses to the large one. It is of interest to point out that the peaks in patient 1 (Figure 5) represent specimens obtained at 9 a.m., whereas the alternate low levels represent specimens taken at 9 p.m. The interval of time from the previous dosage of thiouracil was 4 hours in each instance. We have observed in other cases that the blood level sometimes drops following a meal, particularly the night meal. A possible explanation of this phenomenon is discussed later.

In Figure 6 are plotted the total daily excre-

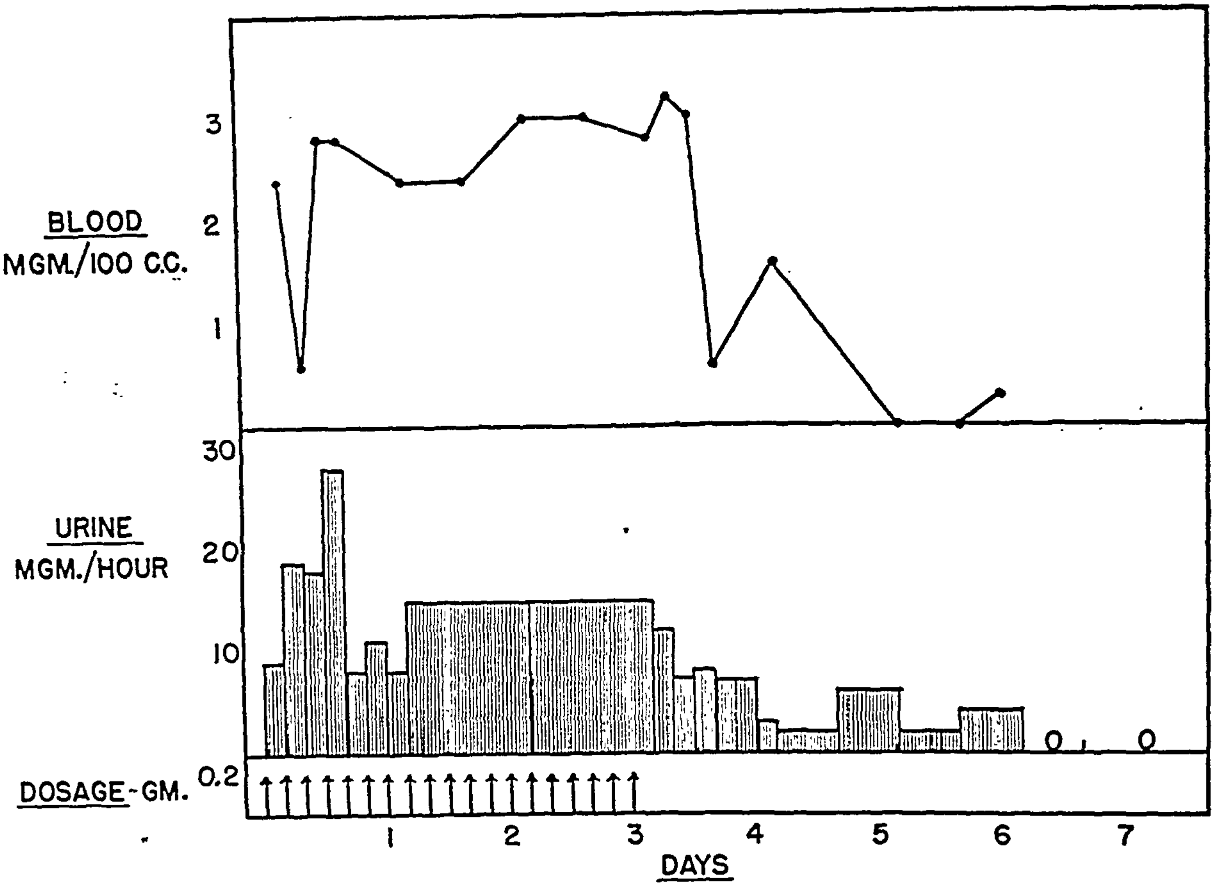

Fig. 3. The Amounts of Thiouracil in the Blood and Urine of a Normal Individual During and Following the Ingestion of 0.2 gram of the Drug Every 4 Hours for 3 Days

tions of thiouracil in the urine of 12 individuals. Subjects 5 and 7 had untreated myxedema, subjects 9 and 10 were normal, and the others had thyrotoxicosis. There was an interval of 24 to 48 hours before the amount of the thiouracil excreted reached a relatively constant level. This was true in the cases receiving the smaller dosages as well as in the cases with the larger dosages. The total excretion in the latter group was greater than in the former; however, the total amount of the drug excreted by any subject was only about one-third of the quantity ingested.

In a few cases, we had the opportunity of following, daily, the changes in the concentration of thiouracil in the blood and urine after discontinuing the administration of the drug. A knowledge of these changes is important in relation to the development of possible complications from this substance and is also of aid in calculating the percentage of the administered drug which is excreted in the urine. One subject who had received 1.2 grams [4] daily for 3 days was found to have a blood level of 1.6 mgm. per 100 cc., 24 hours after discontinuation of the drug. On the following day, none was present. Another patient who had received 1.0 gram daily for 4 weeks was shown to have the following levels on successive days after stopping treatment: 2.5, 0.6, 0.4, 0.0 mgm. per 100 cc. of blood. A third patient who had received 1.0 gram daily for 4 weeks had only traces of thiouracil in the blood 5 days after stopping treatment. A fourth patient, having received 0.8 gram daily for 3 weeks, was found to have no thiouracil in the blood 48 hours after discontinuing therapy. However, in this case, fluids were forced to 5 liters daily.

[4] Throughout this paper, unless otherwise specified, the daily dosage given consists of single doses of 0.2 or 0.25 gram, administered at evenly-spaced intervals throughout the 24-hour period.

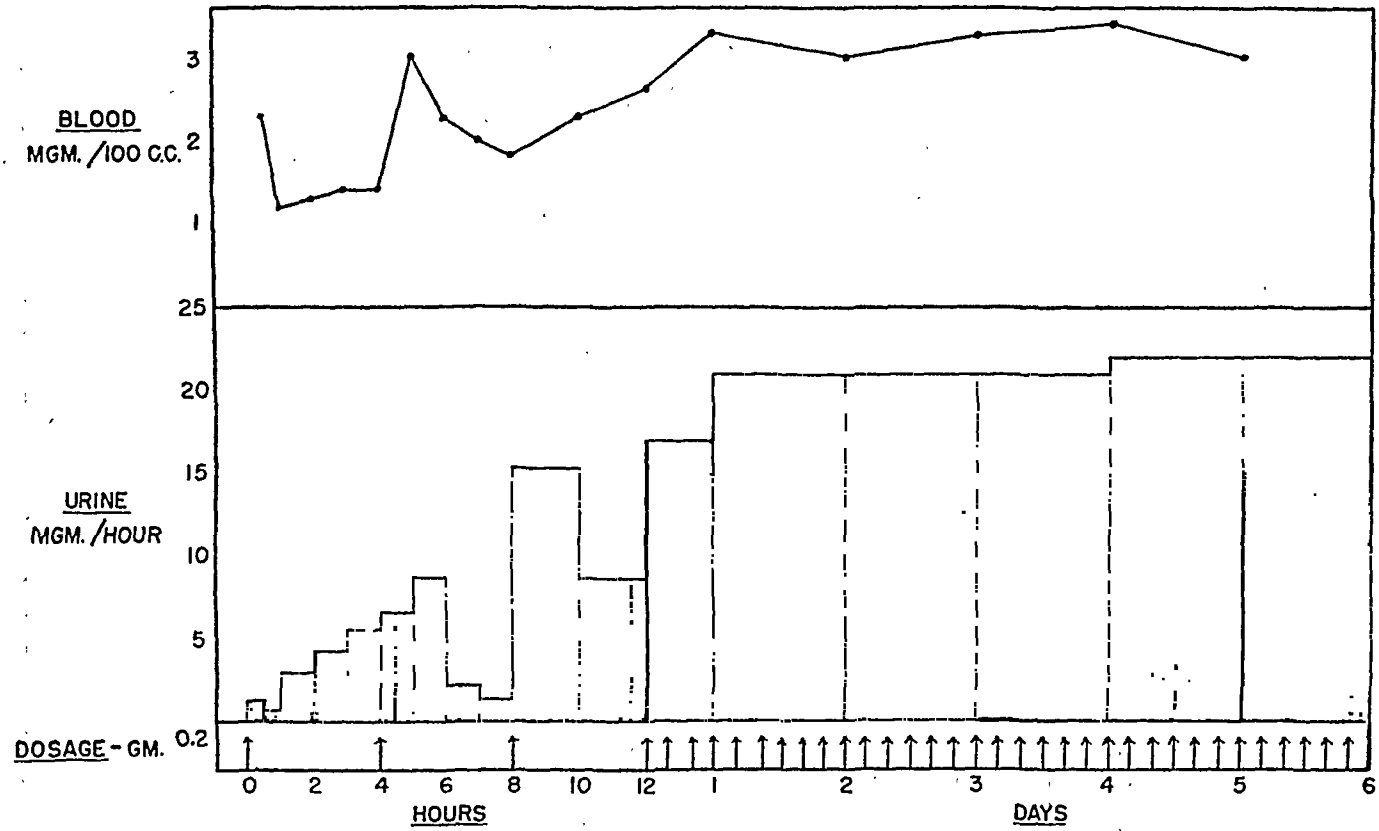

FIG. 4. THE AMOUNTS OF THIOURACIL IN THE BLOOD AND URINE OF AN OBESE INDIVIDUAL DURING THE INGESTION OF 0.2 GRAM OF THIOURACIL EVERY 4 HOURS

Note the constancy of the blood level and renal excretion after the first day.

In 5 patients, we studied the rate of disappearance of thiouracil from the urine, following cessation of treatment. Four of these patients had received 0.8 gram or 1 gram daily for 3 or 4 weeks. The other patient had received treatment for only 3 days, the dosage being 1.2 grams daily. In this patient, all thiouracil had disappeared within 4 days. In the 4 cases receiving the more prolonged treatment, the urine did not become free of thiouracil until from 5 to 7 days. The total amount excreted following discontinuation of the drug just about equaled the usual daily content of the urine. For example, in 1 patient who for several days had excreted about 225 mgm. per day, with the cessation of treatment the daily excretion was as follows: 157 mgm., 33 mgm., 18 mgm., 8 mgm., 8 mgm., 0 mgm. Since the amount of thiouracil excreted in the urine was repeatedly found to be only about one-third of the amount ingested, we investigated the quantity eliminated in the feces. All of the stool specimens of 4 patients receiving 1.2 grams of thiouracil daily were saved for a period of 4 days. None of the drug was recovered from any of the specimens. The failure to find the substance in the stools could be attributed, in large part, to absorption and destruction of the drug high in the intestinal tract, the evidence for which we have given. Nevertheless, the question was raised as to whether colon bacilli break down thiouracil. For this reason, we studied the effects of *E. coli*, staphylococcus aureus, and beta hemolytic streptococcus on this drug. To heavy broth cultures of each of these organisms was added enough thiouracil to make a concentration of 4 mgm. per 100 cc. The mixture was incubated at 37° C. for 24 hours. The colon bacilli caused no destruction of the drug, while the staphylococci caused destruction of 35 per cent, and the streptococci caused destruction of 50 per cent.

In order to estimate the amount of thiouracil broken down in the body in sites other than the gastro-intestinal tract, 500 mgm. of the drug (in the form of its sodium salt) were given in 10 cc. of saline, intravenously, to each of 3 normal sub-

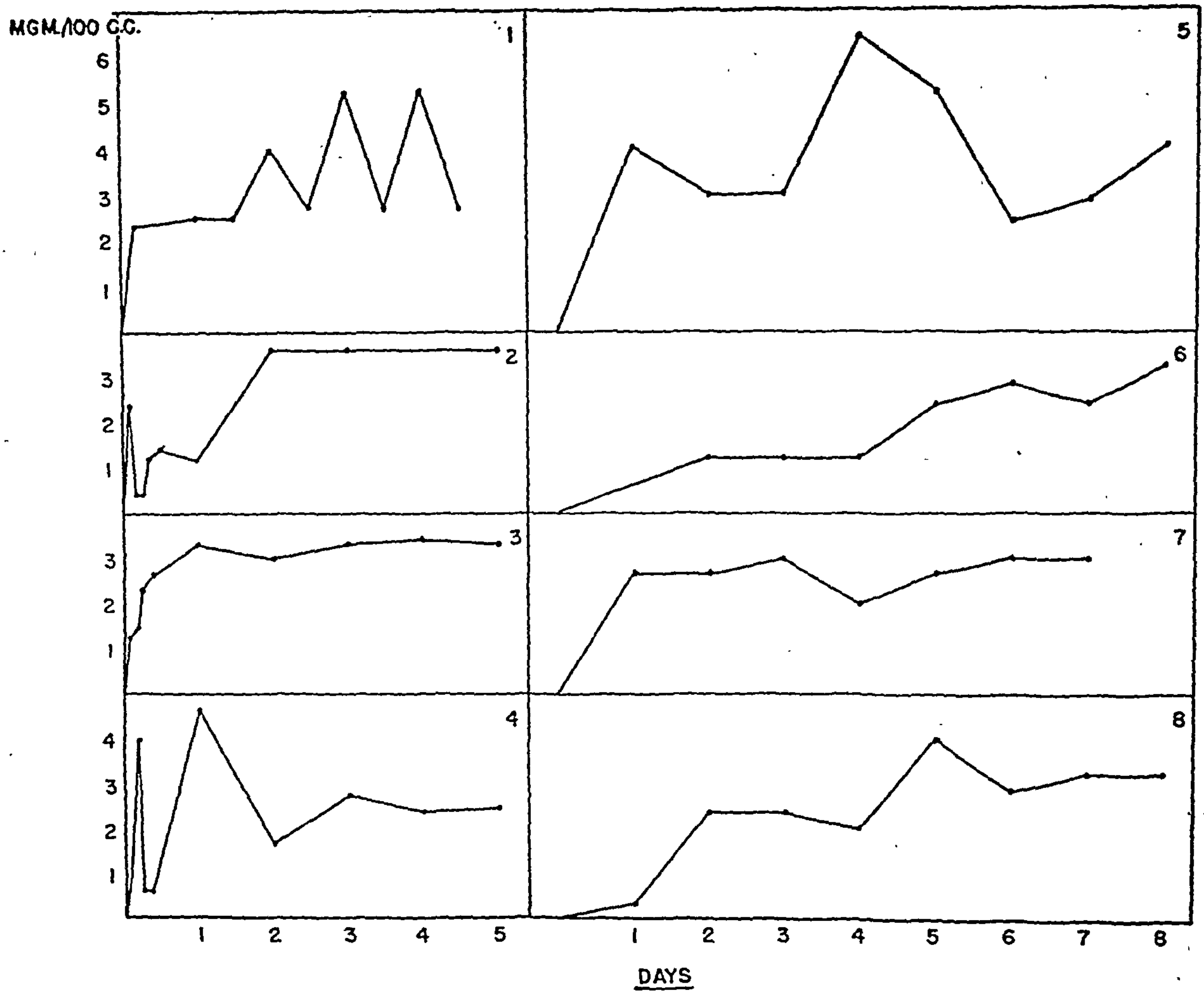

FIG. 5. THE RATE OF ACCUMULATION OF THIOURACIL IN THE BLOOD

The daily dosage for patients 1, 2, and 3 was 1.2 grams; for patients 4 and 5, 0.6 gram; for patients 6 and 7, 0.4 gram; and for patient 8, it was 0.2 gram.

jects. A total of from 41 to 50 per cent of the injected dose was excreted in the urine.

Comparative studies with thiourea have shown that following the injection of 500 mgm. of this substance, intravenously, approximately one-third of the injected dosage is excreted in the urine. The question now arises as to what are the other excretion products of these thiourea substances. Various investigators (5, 6) have found, in man and animals fed thiourea, that almost all of this substance was excreted in the urine in the form of organic sulfur compounds. It seems likely, therefore, that part of the administered thiourea, at least, is transformed in the body in such a way that its sulfur, while still in organic combination, no longer forms a part of the group $\mathrm{N}{-}\overset{\mathrm{S}}{\overset{\|}{\mathrm{C}}}{-}\mathrm{N}$ which gives the color with Grote's reagent, at a pH of from 8.5 to 9. In the case of thiouracil, Mendel and Myers (7) have found that when this substance is fed to dogs, the urine gives a strong qualitative test, which is apparently specific for uracil and cytosine. We are now conducting studies of the specific excretion products of thiouracil which we hope to report at a later date (8).

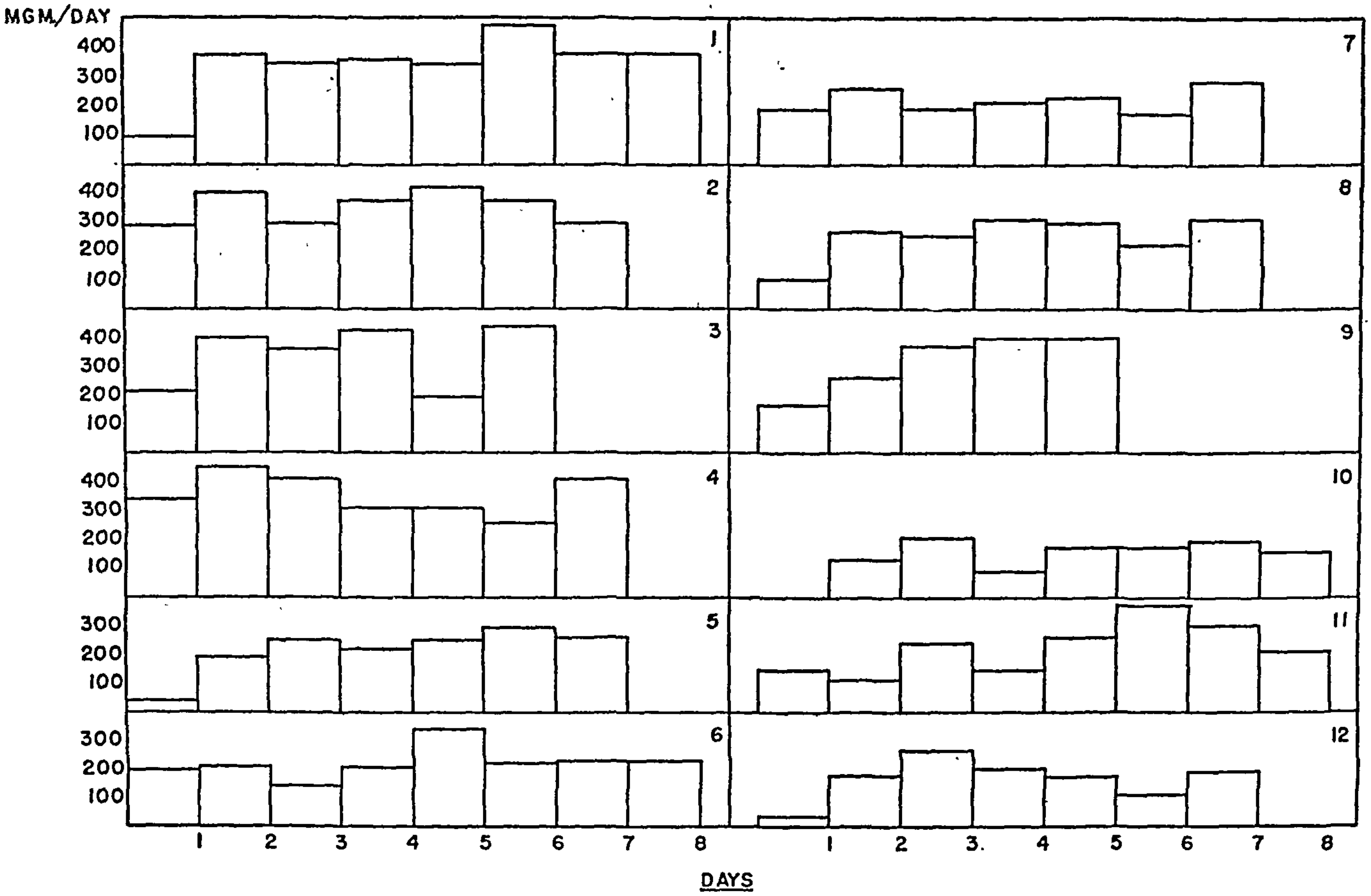

FIG. 6. THE RATE OF EXCRETION OF THIOURACIL IN THE URINE FROM THE BEGINNING OF TREATMENT

The daily dosage for patients 1 to 4 was 1.2 grams; for patients 5 to 9, 1.0 gram; for patients 10 to 11, 0.6 gram; and for patient 12, 0.4 gram.

DISTRIBUTION OF THIOURACIL IN BODY FLUIDS

In a group of patients, mostly thyrotoxics, receiving treatment with thiouracil, we made repeated determinations of the content of this substance in the blood, in order to determine the concentration obtained by various dosages of the drug. A summary of these results is recorded in Table IV. The values given are only those obtained after the patient had been on the stated dosage for more than 3 days. It was believed that, during this interval, the blood level would become relatively stabilized. Although 9 different dosage levels were given, varying from 0.2 to 1.2 grams daily, the average blood level was found to be about 2.5 mgm. per 100 cc. in nearly all. The concentration of thiouracil in the blood was found to vary somewhat in the same individual, although the blood specimen was obtained at the same time of day, with the patient fasting, receiving his medications at regular intervals, and under other such standard conditions on a metabolic ward.

TABLE IV

Concentration of thiouracil in the blood

Daily dosage	Blood level		Number of determinations	Number of patients
	Average	Range		
grams	*mgm. per 100 cc.*			
1.2	3.3	1.2 to 5.2	17	6
1.0	2.5	0.8 to 4.0	12	8
0.8	2.6	2.0 to 2.8	6	4
0.75	2.4	1.2 to 5.2	21	9
0.6	3.0	2.4 to 6.4	17	10
0.5	2.7	1.2 to 5.2	13	6
0.4	2.0	0.8 to 3.2	25	11
0.25	1.8	1.2 to 2.4	11	4
0.2	2.3	1.2 to 4.0	17	6

The distribution of thiouracil in different elements of the blood was studied in 4 individuals who had been treated with this substance for several days (Table V). In 3 subjects, the blood cells were found to contain about 7 times as much as the plasma; in the other subject, the cells contained twice as much as did the plasma. The

TABLE V

Distribution of thiouracil in the blood

Diagnosis		Normal	Obesity	Thyrotoxicosis	Hypertension
Daily dosage of thiouracil (*grams*)		1.0	1.2	1.2	0.5
Thiouracil (*mgm. per 100 cc. of whole blood*)	White blood cells	0.50	0.63	0.92	0.008
	Red blood cells	1.25	2.29	1.83	3.8
	Total cells	2.0	2.98	2.8	3.7
	Plasma	0.29	0.42	0.39	1.8
Hematocrit (*per cent*)	White blood cells	0.1	0.1	0.1	0.1
	Red blood cells	28.5	37.3	35.0	40.0
	Plasma	71.4	62.6	64.9	59.9
White blood cell count		8,950	8,200	6,200	8,200

red blood cells were found to possess 2 or more times the amount present in the white blood cells. However, the average amount of drug per cell was much greater in the white cells than in the red.

Further experiments were conducted on the absorption of thiouracil by the blood cells, *in vitro*. Fifty cc. of blood were obtained from each of 5 patients, of whom one was convalescing from a coronary occlusion, one had obesity, one had acute myelogenous leukemia, one had chronic myelogenous leukemia, and one had chronic lymphogenous leukemia. To the blood were added 10 drops of a 20 per cent solution of potassium oxalate and enough of a 20 mgm. per cent solution of thiouracil to make a final concentration of about 4 mgm. per cent. The mixture was incubated at 38° C. for one hour and immediately thereafter the estimations of thiouracil were begun. It may be observed in Table VI that the higher the white count, the larger the amount of the drug found in the white cells, whether the cells were almost entirely lymphocytes, or whether they were granulocytes. Although the average quantity of thiouracil removed by individual granulocytes was greater than that removed by lymphocytes, the latter cells absorbed more of the drug in comparison to their size. The white cells of the acute leukemic patient removed the same amount per volume of cells as did the chronic myelogenous leukemic cells, but the amount per cell was less in the former group. The red cells of the leukemic pateints did not remove as much thiouracil per volume of cells as did the red cells of the non-leukemic patient. The final concentration of the drug in the plasma was less in the chronic leukemic blood than in the non-leukemic blood. These findings indicate that lymphocytes and granulocytes are very active in ingesting thiouracil from the plasma; the red cells are less active than are the white cells.

The distribution of thiouracil in various tissue fluids was compared with its concentration in the

TABLE VI

Distribution of thiouracil added to blood in vitro

Diagnosis		Obesity	Coronary occlusion	Acute myelogenous leuk.	Chronic myelogenous leuk.	Chronic lymphogenous leuk.
Thiouracil (*mgm. per 100 cc. of whole blood*)	White blood cells	0.5		1.26	3.4	4.0
	Red blood cells	2.1		0.18	0.3	0.5
	Total cells	2.7	1.35	1.59	3.8	4.5
	Plasma	1.5	1.54	2.02	1.0	1.0
	Plasma and cells	4.2	2.89	3.61	4.8	5.5
	Whole blood	4.6	3.12	3.44	4.0	4.6
Hematocrit (*per cent*)	White blood cells	0.2	0.1	3.0	7.6	5.0
	Red blood cells	39.9	40.6	15.0	39.0	30.0
	Plasma	59.9	59.3	82.0	53.4	65.0
White blood count		12,250	7,550	48,000	96,000	160,000

TABLE VII
Thiouracil in body fluids

Type of fluid	Thiouracil in fluid	Thiouracil in blood	Thiouracil treatment	Diagnosis
	mgm. per 100 cc.	*mgm. per 100 cc.*	*grams*	
Cerebrospinal	0.6	2.8	0.4, daily for 22 days	Heart failure
	1.0	1.2	0.5, daily for 10 days	Heart failure
	0.3	1.2	0.6, daily for 7 days	Heart failure
	0	1.2	0.6, daily for 3 days	Convalescent meningitis
	0.7	1.2	1.0, 2 hrs. before	Latent syphilis
	0.5	2.0	1.0, 2 hrs. before	Heart failure
Edema	0.3	3.2	1.0, daily for 3 days	Heart failure
	0.6	1.2	0.6, daily for 7 days	Heart failure
Pericardial	0.3	1.2	0.6, daily for 7 days	Heart failure
Chest	3.0	2.8	0.6, daily for 6 days	Heart failure
	3.2	4.0	0.5, daily for 4 days	Heart failure
	1.0	2.8	0.6, daily for 4 days	Pleural effusion
Ascitic	2.6	2.0	0.75, daily for 7 days	Laennec's cirrhosis
	2.0	2.0	0.75, daily for 4 days	Laennec's cirrhosis
Milk	12.0	4.0	1.0, 2 hrs. before	Postpartum
	9.2	3.2	1.0, 2 hrs. before	Postpartum

blood (Table VII). The pericardial fluid was obtained at autopsy. The edema fluid was collected through a 20-gauge needle inserted into the dorsum of the ankle. The milk specimens were obtained with a breast pump. The cerebrospinal fluid, edema, and pericardial fluids were found to contain distinctly less thiouracil than did the whole blood. However, these fluids possessed about the same concentration of the drug as found in the plasma. The chest fluids and ascitic fluids contained essentially the same proportion of thiouracil as did the blood; however, milk was found to have 3 times as much.

The daily excretion of thiouracil in the urine was estimated with a group of patients, mostly thyrotoxics, treated with dosages ranging from 0.2 to 1.2 grams daily (Table VIII). The amount of the drug in the urine was, in general, about one-third of the total amount ingested; however, there was moderate variability in the quantity excreted by different individuals maintained on the same dosage.

Three patients with nephritis were studied in order to observe the effect of impaired kidney function on the excretion of thiouracil and on its accumulation in the blood. One patient with chronic glomerular nephritis and marked uremia was given 0.75 gram of the drug for 10 days, during the last 3 of which all urine was saved for analysis. During this time, the average daily amount of the drug excreted was 3 mgm., whereas in normal subjects with comparable treatment, the excretion is 100 times this amount. However, in spite of the failure of the nephritic patient to excrete the drug, the blood level did not exceed the normal range. Another patient with chronic glomerular nephritis and uremia, who was treated with 0.75 gram of thiouracil daily, excreted an average of 24 mgm. per day during a period of 7 days. As in the first case, the blood level remained normal. In a third patient with healed pyelonephritis and only moderate impairment of renal function, the average daily

TABLE VIII
Excretion of thiouracil in the urine

Daily dosage	Thiouracil in urine		Number of determinations	Number of patients
	Average	Range*		
grams	*mgm. per day*			
1.2	373	150 to 618	49	8
1.0	249	75 to 482	50	8
0.8	299	115 to 448	3	2
0.75	324	218 to 388	6	2
0.6	206	87 to 382	16	2
0.5	96	65 to 152	6	2
0.4	149	55 to 265	12	5
0.25	101	84 to 132	6	2
0.2	82	16 to 202	13	2

* The values given include only the specimens obtained after the patient had received treatment for 3 days or more.

excretion of thiouracil was only about 25 per cent of normal, but the blood level remained essentially normal. These observations indicate that the drug may be broken down by the body tissues. Our attention was first directed to the liver as possibly being the chief site for the breakdown of the drug. If such is the case, one might expect subjects with severe liver disease to show a greater rise in the thiouracil level of the blood than is shown in normal subjects. Two patients with severe cirrhosis were given 0.75 gram of thiouracil daily. All urine was saved and frequent blood specimens were obtained. However, the amount of the drug in the urine and the blood was repeatedly normal in each patient. The question then arose as to whether many tissues participated in the breakdown of thiouracil. Thereupon, we investigated the effect of isolated tissues by adding known amounts of the chemical to tissue slices in Warburg vessels.

DESTRUCTION OF THIOURACIL BY TISSUE SLICES

From 30 to 80 mgm. of rat liver, in the form of thin slices, were placed in each of 5 test tubes containing 2.7 cc. of a phosphate salt medium. To this was added 0.3 cc. of a solution containing 100 mgm. per cent of thiouracil. The tubes were shaken in a water bath at 38° C. for varying intervals and then the remaining quantity of thiouracil was estimated in the tissue, as well as in the medium. Within 2 minutes, none of the drug was destroyed, but in 10 minutes, there was 14 per cent destruction; in 30 minutes, 17 per cent; in 1 hour, 20 per cent; and in 2 hours, 33 per cent.

In a series of experiments, we studied the breakdown of thiouracil produced by slices of kidney, pituitary, thyroid, adrenal, pancreas, and striated muscle. In the first experiment, thin slices of tissues, totalling in wet weight from 3 to 6 mgm., were placed in test tubes containing 5 cc. of phosphate medium and 0.48 mgm. of thiouracil. The tubes were shaken in a water bath at 38° C. for 2 hours, during which time oxygen was passed over the surface of the medium. The pH of the solution was 7.4 at the beginning of the experiment and essentially the same at the end. At the end of the experiment,

TABLE IX

Destruction of thiouracil by tissue slices

Experiment		Wet weight*	Destruction	Thiouracil destroyed per gram of tissue
		mgm.	*per cent*	*mgm.*
No. 1	Kidney	67	47	3.4
	Pituitary	3	13	27.0
	Thyroid	14.8	72	23.7
	Adrenal	36.8	72	9.3
	Pancreas	61.1	5	0.4
	Muscle	62.2	2	0.1
No. 2	Kidney	91.2	83	4.4
	Liver	109.2	90	3.9
	Pituitary	7	61	41.3
	Thyroid	24.7	50	10.1
	Adrenal	66.6	84	6.2
	Pancreas	172.8	39	1.1
	Muscle	79.4	43	2.5
No. 3	Kidney	74.9	42	2.8
	Liver	187.1	3	0.1
	Pituitary	1.2	76	316.0
	Thyroid	18.9	73	19.0
	Adrenal	70.6	22	1.6
	Pancreas	276.1	21	0.4
	Muscle	155.3	14	0.5
No. 4	Liver	13.4	89	29.8
		37.4	61	7.2
		77.3	17	0.6
		198.6	16	0.2
No. 5	Liver	7.6	86	48.6
		41.0	65	7.2
		89.6	15	0.9
		191.2	12	0.3
		384.4	3	0.03

* Wet weights were used rather than dry weights because drying at 100° C. causes destruction of thiouracil.

the amount of thiouracil remaining in the medium and the tissues was determined. Two subsequent experiments were conducted using liver slices in addition to the others. These 2 experiments differed from the first in that Warburg vessels were used instead of test tubes, larger quantities of tissues were employed, and 0.50 mgm. of thiouracil was added. All of the tissues were active in destroying the drug (Table IX); in some instances, more than 75 per cent was destroyed. Less than 5 per cent of the drug was found in any of the tissues. When the amount of destruction of thiouracil was calculated on the basis of 1 gram of tissue, it was found that the pituitary tissue was the most active, while the thyroid and adrenal were next most active; muscle and pancreas were least active. How-

ever, it soon became apparent that within certain limits, the more tissue present, the less was the total destruction. This apparent paradox was investigated in the case of liver slices (Experiments 4 and 5, Table IX). To a series of Warburg vessels containing 5 cc. of phosphate medium and 9 mgm. per cent thiouracil, were added thin slices of liver in widely varying quantities. The mixture was shaken in a water bath at 38° C. for 2 hours. When 7.6 mgm. of liver slices were used, there was a destruction of 86 per cent of the thiouracil. With an increase in the amount of liver, there was a progressive decrease in the absolute quantity of drug destroyed, the destruction being only 3 per cent with 384 mgm. of liver. No destruction took place in the control flask, which contained the same constituents as the others, with the exception of the liver slices. A more extensive report on this phenomenon will be made later.

DISTRIBUTION OF THIOURACIL IN TISSUES

Autopsy specimens

A group of patients with various diseases, bearing an essentially hopeless prognosis, were given thiouracil for one or more days preceding death. At autopsy, specimens were obtained from most of the tissues of the body. In the case of small organs, such as the adrenal and pituitary, essentially all of the tissue was used in the preparation of a fine suspension; with the larger structures, about 100 grams were used. Excess blood was squeezed out and washed from the surface and the tissue was then analyzed for its content of thiouracil by the method which is given in a separate report (1).

In conducting such a study, we had hopes of demonstrating in a crude manner the relative concentrations of the drug throughout the various tissues of the body. In interpreting the results, we have borne in mind the fact that all of the subjects studied were markedly abnormal in many ways. In most of the patients, there probably was an impairment in the absorption, distribution, and excretion of the drug. Furthermore, there were variations in the total dosage of the drug and in the interval from death until the analysis of the tissue. The data presented in the previous section suggest that some destruction of the drug might take place immediately

TABLE X

Distribution of thiouracil in tissues *

Patient	C. O.	M. A.	R. Y.	M. A. T.	W. R.	C. R.	R. I.	H. O.	Q. U.	M. O.
Age (years)	60	72	54	60	24	60	65	60	69	73
Cause of death	Pneumonia, pyelonephritis	Heart failure	Heart failure	Uremia	Acute lymphatic leukemia	Cerebral hemorrhage	Pulmonary tuberculosis	Uremia	Bacteremia, uremia	Cerebral hemorrhage
Hours post-mortem	3	2	14	17	8	4	336	12	13	29
Daily dosage (*grams*)	0.4	0.5	0.6	0.6	0.6	1.2	0.75	0.5	1.0	1.2
Total dosage (*grams*)	8.8	5.0	4.2	3.6	3.6	3.4	2.5	0.5	1.0	2.2
Pituitary	69.9		22.9	25.8	118.0	26.0		5.7	19.5	3.2
Adrenal	41.0	83.4	21.7	35.6	47.3	28.3	8.5	9.2	11.7	2.1
Thyroid	41.6	152.0	41.2	15.8	214.0	1.9	5.3	2.0	0.2	2.9
Pancreas	2.7	65.0	31.1	11.8		2.2				1.7
Testes	23.4		19.3	14.6		2.8	0		0	
Ovaries		209.0			65.1					1.5
Bone marrow	115.0	435.0	50.3	40.6	204.0	16.4	65.2		11.2	2.6
Kidneys	78.9	97.0	11.7	19.5	49.5	1.5	0		0.2	1.1
Liver	6.4	12.2	18.9	12.2	21.2	2.8	1.8	1.0	0.2	3.9
Spleen	42.1	38.6	11.2	8.8	37.7	1.0			2.7	1.4
Muscle	4.7	6.5	0	0	8.2	3.0			0.4	0.6
Heart						1.1			1.6	0.4
Lungs						2.4			0.9	0.4
Brain	45.8			6.7		2.2			0.5	3.4
Prostate			0			3.8			5.4	
Blood (*mgm. per 100 cc.*)	2.8	1.2	1.2	2.8	2.8		1.0			

* The concentration of the drug in the tissues is expressed in terms of mgm. per 100 grams (dry weight) of tissue. With 3 cases, the fat-free dry weight was determined for each of the tissues but these values correlated so closely with the dry weight that this measure was discontinued.

TABLE XI

Distribution of thiouracil in goiters

Patient	M. K.	B. O.	C. A.	M. G.	G. O.
Diagnosis	Non-toxic nodular goiter	Non-toxic nodular goiter	Papillary cystadenoma	Toxic nodular goiter	Toxic diffuse goiter
Daily dosage (*grams*)	0.75	0.6	0.6	0.75	0.5
Days treated	12	10	10	9	3
Relatively normal thyroid tissue*	0.7	3.1		4.4; 3.9	0.3; 0.3
Adenomatous thyroid tissue*	6.8	20.6	16.3		
Blood level (*mgm. per 100 cc.*)	2.4	3.2	3.0	3.0	0.4

* Mgm. thiouracil for 100 grams (dry weight) of tissue.

after the death of the patient. Frequent analyses were made of the thiouracil content of a diffusely hyperplastic thyroid during an interval of 4 hours following thyroidectomy, but no changes were noted. Similar studies conducted with guinea pig livers and kidneys have shown essentially no changes during the first 2 hours after death of the animal but slight changes thereafter. Once alkaline extracts of the tissue are prepared (1), no change in the thiouracil content has been found to occur during a period of several days.

Almost all of the specimens examined (Table X) were found to contain thiouracil. In the 5 cases dying within 3 days from the first dose of thiouracil, the greatest concentration of the drug was found in the pituitary, adrenals, bone marrow, and thyroid. In some of the 5 cases receiving thiouracil for 6 days or more, large quantities of the drug were concentrated in the bone marrow, thyroid, ovaries, and pituitary. The adrenals, kidneys, spleen, and pancreas also possessed a relatively large amount, but the muscle, liver, and testes contained a distinctly smaller quantity. In the patients who received treatment for more than 5 days, it was found that the concentration of the drug in most of the tissues was many times its concentration in the blood, in a few specimens being more than 50 times as great.

Surgical specimens

One patient who had been treated for thyrotoxicosis with thiouracil developed attacks of gall bladder colic which was found to be due to gall stones. At the time of the gall bladder operation, a small piece of liver and of skeletal muscle were removed for analysis of the thiouracil content. She had received a total of 17.8 grams of thiouracil during a period of 27 days. The liver was found to contain 23.9 mgm. of the drug per 100 grams (dry weight) while the muscle contained 11.5 mgm. per 100 grams. Therefore, the concentration in each tissue was greater than was found in any of the autopsy specimens. Several factors may perhaps be involved to account for the difference; namely, the better metabolic condition of the patient, longer period of treatment, and shorter period of time for the destruction of the thiouracil by the tissue (analysis immediately after excision of the tissue).

Five patients with goiters were given from 0.5 gram to 0.75 gram of thiouracil for from 3 to 12 days preceding thyroidectomy and the tissue removed was analyzed for the content of the drug. Three of the patients had non-toxic nodular goiters while 2 had toxic goiters. Each of the patients with toxic goiters had received treatment with potassium iodide for several weeks and their basal metabolic rates were normal, both at the time that thiouracil was begun and at the time of the operation.

The gland of one patient (M. K., Table XI) contained several nodules, one of which was grayish red, quite cellular, and friable; the other nodules were beefy red, contained a slight excess of colloid and a few strands of fibrous tissue. Microscopic examination revealed that the grayish-red nodule consisted of adenomatous tissue (Figure 7A) whereas the remainder of the gland was essentially normal (Figure 7B). The adenomatous tissue was found to have a concentration of thiouracil 10 times that present in the remainder of the tissue.

Patient B. O., who also had a non-toxic goiter, was found to have an adenoma of the thyroid.

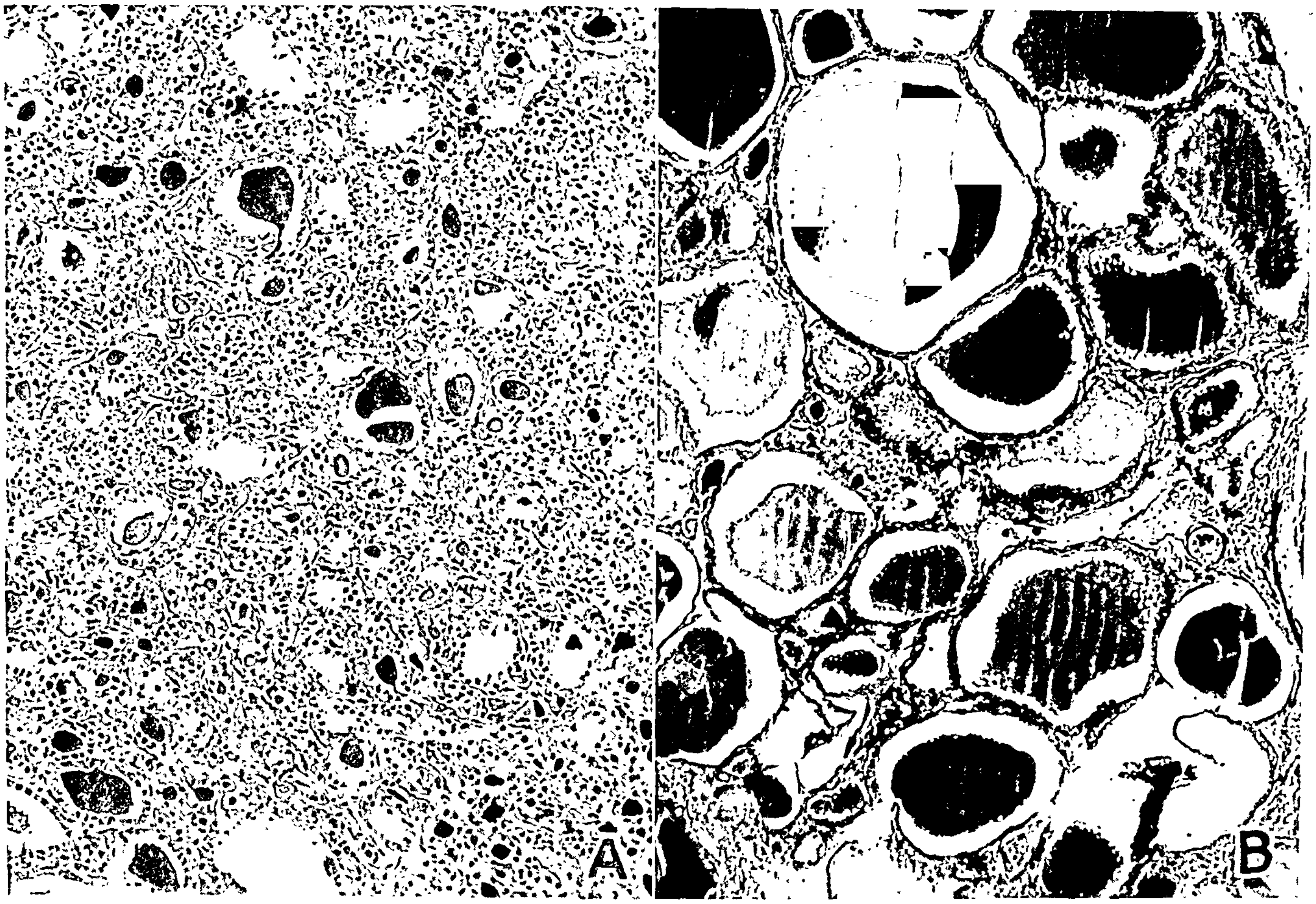

FIG. 7. SECTIONS OF A NON-TOXIC NODULAR GOITER (PATIENT M. K., TABLE XI). A. ADENOMATOUS TISSUE. B. RELATIVELY NORMAL TISSUE

The remainder of the gland contained a few scars, an increase in cell height in a few areas, and had slightly less colloid than normal. The adenomatous tissue was found to have a concentration of thiouracil which was 6 times that of the relatively normal tissue.

From patient C. A. was removed a papillary cystadenoma. No normal tissue was removed for comparison, but the concentration of the drug in the adenoma was similar to that in the adenoma of the previous patient.

Patient M. G. had a toxic nodular goiter. The tissue looked very much the same throughout, macroscopically and microscopically. The epithelium was flat and there was a slight excess of colloid. Two different samples of the gland were found to have essentially the same concentration of thiouracil, 4.4 and 3.9 mgm. per cent (dry weight of tissue).

Patient G. O. had a toxic goiter with diffuse hyperplasia. Microscopically, the gland was essentially normal. In each of 2 portions of the gland, the concentration of the drug was 0.3 mgm. per 100 grams (dry weight).

SUMMARY

1. Thiouracil is very rapidly absorbed from the gastro-intestinal tract and is readily excreted in the urine.

2. With dosages ranging from 0.2 to 1.2 grams daily, the concentration of the drug in the blood varied from 0.8 to 6.4 mgm. per cent, while the daily excretion in the urine varied from 16 to 618 mgm.

3. Most of the thiouracil in the blood is in the cells, the red cells containing a larger total amount but smaller concentration than the white cells.

4. Patients receiving the drug for several days preceding death were found at autopsy to have some of the substance in essentially all of the

tissues of the body. Thiouracil was sometimes found in very large quantities in the bone marrow, thyroid, ovaries, and pituitary, while striated muscle, testes, and liver possessed relatively small concentrations.

5. Adenomata of the thyroid gland possessed a much greater concentration of thiouracil than did relatively normal thyroid tissue.

6. Cerebrospinal, edema, and pericardial fluids were found to contain distinctly less thiouracil than did whole blood; the concentration in pleural and ascitic fluid was about equal to that of blood, whereas milk contained about 3 times as much.

7. Thiouracil is rapidly destroyed by the contents of the stomach and the small intestines. It is also rapidly destroyed by many tissues of the body.

8. No thiouracil is excreted in the stools. The colon bacillus does not account for its absence since it does not destroy the drug.

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SIMULTANEOUS MEASUREMENTS OF THE BLOOD VOLUME IN MAN AND DOG BY MEANS OF EVANS BLUE DYE, T1824, AND BY MEANS OF CARBON MONOXIDE. I. NORMAL SUBJECTS[1]

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The volume of the circulating blood may, in principle, be estimated indirectly by determining the apparent volume of distribution of any substance which, upon being introduced into the circulation, does not escape from the vascular bed. All substances which have proven in any way suitable for this purpose fall into one or another of two main classes. Those of one group, such as certain dyes, are distributed mainly through the plasma, and measure plasma volume only. The most desirable dyes, such as T1824, are attached to the serum albumin (1). Those of the second group, such as carbon monoxide and radioactive iron (2), are confined mainly within the erythrocytes. This second group of substances therefore measures, primarily, cell volume. Although these substances are distributed through entirely different compartments of the total blood volume, it is possible to calculate the plasma volume from the cell volume and *vice versa*, if the fraction of cells in the circulating blood is simultaneously determined. It is therefore possible to measure whole blood volume by either method, and to compare the results. Were all assumptions implicit in their use correct, identical values for cell and plasma volumes should result. Any discrepancy between the values obtained by these two methods necessarily means that the assumptions underlying at least one of these methods are in error. On the other hand, the reverse proposition does not hold; to obtain identical numerical results by the two methods does not necessarily imply that they are both measuring true blood volume.

In the present study, simultaneous estimations of blood volume by the blue dye, T1824,[3] and by carbon monoxide have been made in the dog and in man. The third variable, relative red blood cell volume, was measured by means of the hematocrit. Only results obtained in normal subjects are included in this paper; those in pathological subjects are presented in the following paper (3). Such simultaneous or nearly simultaneous measurements have only rarely been made (4 to 10), and very seldom with satisfactory modern technics. Yet coincidence of measurement is an absolute necessity if anything but a rough comparison of the two methods is to be attempted, since blood volume may change distinctly in the time elapsing between successive determinations. Temperature change, variation in the degree of physical relaxation, and a variety of nervous and hormonal effects are all known to influence blood volume markedly. Simultaneous measurement obviates difficulties due to the interplay of these influences.

MATERIAL AND METHODS

The group of human subjects studied was made up almost entirely of laboratory technicians, medical students, and physicians. All had normal blood pressures and normal serum protein concentrations. All subjects were in a post-absorptive state, as nearly basal as circumstances would allow. They were recumbent during the experiments.

The dogs were of mixed breed, in a good state of nutrition, and all but 2 were females. The splenectomized animals were not used until at least 2 or 3 weeks after the operation. Studies were done from 12 to 18 hours after the last feeding. Experiments were conducted with the animal upon its back strapped to an animal board. Some of the animals (Table III) were anesthetized with "Dial," 0.6 cc. per kgm., and a tracheal cannula inserted through

[1] Aided by a grant from the Fluid Research Fund, Yale University School of Medicine, and from the Ella Sachs Plotz Fund.

[2] Alexander Brown Coxe Fellow, 1941–1942, and American College of Physicians Fellow 1942–1943.

[3] The blue dye, T1824, was furnished by the Warner Institute for Therapeutic Research, New York City.

which carbon monoxide was administered. It is to be noted that the effect of "Dial" on the spleen (11, 12) would not invalidate a comparison of the two methods done simultaneously. A constant temperature room was not used; however, room temperature was always recorded, and rarely varied more than 1 or 2 degrees during any single experiment.

Fasting samples for dye and carbon monoxide blanks and for hematocrit determinations were always taken initially. The dye was then given. Ten minutes later, a blood sample was taken for determination of the dye. The carbon monoxide was usually given immediately after the taking of this sample, although sometimes it was given immediately after giving the dye. A blood sample was taken for carbon monoxide analysis, 15 to 20 minutes after beginning the inhalation of the carbon monoxide. Further hematocrit determinations were frequently made.

Relative cell volume was measured in quadruplicate upon anaerobically defibrinated blood, by a method described by Eisenman (13), using calibrated Daland microhematocrit tubes. This method of defibrinating blood avoids the error produced by various anti-coagulants which may cause swelling or shrinkage of cells. The results are entirely reproducible, and apparently give the true relative cell volume, as indicated by Eisenman, MacKenzie, and Peters (14).

USE OF DYE

Plasma volumes were determined by the method of Gibson and Evelyn (15), using an Evelyn micro-photoelectric colorimeter. Readings were always made with serum at a depth of 10 mm. The "K" values and the "L540, L620" ratios for the lot of dye and for the particular machine used were determined (15, 16). All serum samples were examined, using first a 620 and then a 540 filter; whenever hemolysis was found, a correction was applied, as suggested by Gibson and Evelyn (15). In man, hemolysis was very rare; in the dog, not uncommon. If hemolysis was excessive, the entire blood volume study was discarded.

The precautions used in the handling of blood have been in part described elsewhere. Blood was taken with minimal stasis into oiled syringes. Serum samples for the determination of dye were spun twice, separated, and read in the colorimeter as soon as possible after the blood was drawn, usually within 1 hour. Phillips (17) has observed that under certain circumstances, dyed serum becomes decolorized on standing; therefore, it is desirable not to allow it to stand.

Ten minutes were allowed for mixing of the dye (33). Although disappearance curves were determined in many instances, all plasma volumes here given are, in the interest of simplicity, arbitrarily calculated from the concentration of the dye found in the 10-minute sample. Extrapolation of dye concentration back to the time of injection was not attempted. The uncertainty in distinguishing between mixing time and effects of dye loss makes this procedure also an arbitrary one.[4] Therefore, it is not clear that extrapolation results in any greater precision of measurement.

It should be clearly understood that this single point method of calculating blood plasma volume necessarily always gives larger plasma volumes than do extrapolation methods, since the latter use a calculated figure for initial concentration of dye which is higher than the measured concentration after 10 minutes. This systematic difference amounts to some 5 to 10 per cent, and probably accounts for the fact that our values for plasma volume by the dye method slightly exceed those obtained by Gibson and Evans (19).

In some cases, multiple injections of dye were given. Under these circumstances, the presence of the residual dye was compensated for by the method of Gibson and Evelyn (15). A center setting was obtained, using the initial sample containing only old dye remaining from the preceding tests. The samples containing new as well as old dye were read against this center setting as a blank. The objection by Price and Longmire (16), that this method does not take into account continuing disappearance of original dye, becomes unimportant when only a brief 10-minute mixing time is used, since there is little further loss of the original dye in this time.

A single lot of 0.2 per cent stock T1824 in 0.85 per cent saline and a single calibrated syringe were used for all of the determinations. A 10 cc. aliquot of dye was injected during 30 or 40 seconds; blood was drawn back and forth into the syringe 2 or 3 times in order to insure complete delivery of the dye. If it was desired to give less dye, an initial dilution with physiological saline was made. The error in delivery approximated as much as 0.1 cc., representing a 1 per cent error. In dogs, the dye was always given into a dorsal forearm vein, where extravasation was readily detected. Blood for the determination of dye concentration was always taken with a needle other than that with which the dye was given, at a site remote from the site of injection. In human subjects, it was always

[4] King, Cole, and Oppenheimer (18) suggest a method for plotting the curve of dye disappearance based upon the fact that the loss of dye is proportional to the square root of time after injection. This method of plotting dye disappearance gives a somewhat more precise mathematical definition to the location of the extrapolated curve than does the linear extrapolation method. However, this square root extrapolation method of plotting dye concentration, like any other extrapolation method, depends for its validity upon the assumption that the loss of dye during the mixing time can be determined from a study of the later part of the disappearance curve. This assumption is highly conjectural, as is the assumption that the dye is not distributed into a space much larger than the vascular compartment, even during mixing time. This problem is further considered elsewhere (3). We therefore believe that the use of the dye concentration after 10 minutes gives results at least as accurate as those obtained by any of the extrapolation methods, and avoids the introduction of unwarranted assumptions.

taken from the opposite arm; when repeated samples of blood were taken, the needle was left in place, as described by Gibson and Evans (19).

Carbon monoxide was not found to produce any discernible effect upon the dye disappearance curves, whether given before or after the 10-minute dye sample. Also, the color of plasma containing no dye was found to be unaffected by the giving of carbon monoxide.

USE OF CARBON MONOXIDE

In the course of preliminary experiments with technic, direct intravenous administration of carbon monoxide was tried both in dogs and in human beings. Van Slyke (20) mentions that it may be given intravenously, but apparently this mode of administration has seldom been attempted, although recently oxygen has been given in this manner (21). It was hoped that intravenous administration might be of especial advantage in animals and in patients, such as severe cardiac subjects, who for some reason could not tolerate a mask. Intravenous administration, however, did not eliminate the need for a mask since large amounts of carbon monoxide almost immediately appeared in the expired air. It was, therefore, necessary to use a mask and a system for collecting all expired air, in order to measure the amount of carbon monoxide lost by this route. As much as 20 per cent of the carbon monoxide may be lost by way of the lungs during the process of administration. Also, too rapid administration results in discomfort to the patient, causing both coughing and substernal pain. With slow administration, these symptoms did not occur. Work on this method of administering carbon monoxide is continuing.

The carbon monoxide method for determination of blood volume used in the present study consists of (*a*) administration of a small, measured non-toxic amount of carbon monoxide to the subject by means of a closed respiratory system, and (*b*) measurement of the change in concentration of carbon monoxide in the blood stream. The blood volume (*c*) is then estimated from the extent to which the absorbed carbon monoxide is diluted.

(*a*) *Administration of carbon monoxide*

Measurement of the amount of carbon monoxide absorbed requires a knowledge of the purity of stock carbon monoxide, of the exact amount of this gas introduced into the closed respiration system, and of the amount of unabsorbed carbon monoxide left in the system after rebreathing.

Purity of stock carbon monoxide. The oxygen and carbon dioxide in stock gas were measured by means of a Haldane apparatus. From the volume of oxygen, the volume of nitrogen was estimated on the assumption that the only contaminant was room air containing these gases in the usual proportion. The contaminating air can be measured with an error not exceeding 0.002 cc. in 10 cc. of total gas.

Carbon monoxide gas was prepared by the action of sulfuric acid on formic acid (22). The carbon monoxide was first passed through a 10 per cent solution of sodium hydroxide to remove carbon dioxide and sulfides. It was then stored under positive pressure over 5 per cent sodium hydroxide, free of carbon dioxide. The pressure bottle was kept filled to the top with the alkaline solution covered by a layer of oil and was stoppered when not in use. Stored in this way the gas retained its purity well, changing by no more than 1 per cent per week.

Measurement of the gas administered. The apparatus for the administration of carbon monoxide was essentially that of Chang and Harrop (23). It consisted of a 5 liter rubber bag with 2 openings, a narrow one for the introduction of carbon monoxide and oxygen from the burette, and a wide one connected to a 500 cc. soda lime canister. The canister in turn was packed in ice to prevent undue heating, and was connected by a short wide tube to the mouthpiece. Just before the rebreathing period, a noseclip was applied. The small volume of this apparatus favored the absorption of carbon monoxide, since the absorption of carbon monoxide in the lungs is facilitated by a low oxygen tension (24). Carbon monoxide was introduced into this system from a water jacketed burette, with an error in measurement not exceeding 1 part in 500.

Measurement of unabsorbed carbon monoxide after rebreathing. The amount of carbon monoxide left in the apparatus was quite variable, but was often a significant fraction of the amount originally introduced. The concentration of carbon monoxide remaining in an aliquot of gas was determined by the palladium method of Wennesland (25). Recovery by this method is not quite complete (25, 26), but the error does not exceed 5 per cent. The maximum error would not change the amount of residual carbon monoxide by more than 0.1 cc., which in turn corresponds to the insignificant error of 1 part in 1000 in the measurement of the total carbon monoxide absorbed. The cause of this low recovery is obscure; it is not due to loss of carbon monoxide in the interval between addition of sulfuric acid to blood and the coupling of the flasks. The rebreathing bag and pulmonary space were assumed to have a volume of 4500 cc. in man (23) and 2500 cc. in the dog. Total residual carbon monoxide was obtained by multiplying the concentration of gas by this volume. A considerable error in the estimation of residual carbon monoxide results in much less of an error in the estimation of blood volume. For example, the maximum error in the measurement of blood volume due to differences among triplicate chemical analyses of the residual gas was 0.4 ± 0.5 per cent in 12 sets of analyses in human subjects and 1.53 ± 0.41 per cent in 53 sets of analyses in dogs.

(*b*) *Measurement of change in concentration of carbon monoxide in blood*

Blood for analysis was defibrinated anaerobically over mercury (13). Two methods for the measurement of carbon monoxide content were used, that of Sendroy and Liu (27) and that of Wennesland (25). The latter depends on the ability of carbon monoxide to reduce palladium chloride. The error of this palladium method, in 78 sets of triplicate analyses of blood obtained after carbon monoxide inhalation, was 0.060 ± 0.040 volumes per cent of gas; the greatest individual difference from the average of the 3 determinations was 0.034 ± 0.025 volumes per

cent. The error in 46 sets of triplicate analyses of blood taken before inhalation of carbon monoxide was almost the same, 0.045 ± 0.044 volumes per cent of carbon monoxide; the greatest single difference from the average of 3 was 0.023 ± 0.015 volumes per cent. Since, therefore, the error in the palladium method is an absolute one, the percentage error decreases with increasing concentration of carbon monoxide.

In Table I, 13 sets of simultaneous analyses with both methods are compared. The palladium analyses were done in triplicate, those by the method of Sendroy and Liu either in duplicate or in triplicate. The 2 methods in our hands evidently gave substantially equal results. This is contrary to the experience of Wennesland, who failed to obtain as complete recovery of carbon monoxide from blood with the palladium method as with that of Sendroy and Liu. To make the values equal, he applied a constant correction factor of 1.04 to his palladium figures. From his own data, however, there is evidence that the proper factor is not a constant but a variable, and that at low concentrations of carbon monoxide, it is unity. In his Table VI, only 1 of 4 analyses of 2 blood samples with a low carbon monoxide content (samples 3 and 4) is improved by the use of his correction factor of 1.04; the other 3 are either not improved or are made worse (Samples 1 and 2 are admittedly inaccurate because of excess agitation). With blood specimens containing more carbon monoxide, the correction factor of 1.04 is approximately correct; in 242 determinations (Table III), the average correction factor is 1.0375, and in 113 subsequent analyses (p. 63), the average correction factor is 1.043. Analyses of air samples containing even larger amounts of carbon monoxide, equal to that in blood samples half saturated with gas, require the still larger average correction factor of 1.508. His blood samples usually contained more carbon monoxide than did ours. This fact offers a possible explanation of the discrepancy between his results and ours,

TABLE I

Comparison between Sendroy method and palladium method on the recovery of carbon monoxide from blood

Subject	Date 1942	Carbon monoxide, volumes per cent	
		Palladium method	Sendroy and Liu method
Dog 11	Sept. 24	2.47	2.49
J.J.W.	Sept. 28	1.66	1.70
D.G.	Oct. 5	1.97	1.93
P.H.	Oct. 7	3.07	3.03
B.K.	Oct. 8	2.02	1.93
E.R.	Oct. 9	2.13	2.15
Dog C	Oct. 13	3.64	3.62
D.W.S.	Oct. 14	1.76	1.76
M.S.	Oct. 16	2.54	2.51
M.O.	Oct. 21	2.30	2.32
S.J.	Oct. 22	1.82	1.81
C.B.	Nov. 16	2.08	2.09
H.S.	Nov. 9	3.22	3.22
		Average 2.36	Average 2.35

if we assume that the low range of concentrations in our experiments is correlated with a correction factor too small to be significant.

(c) Procedure and calculation of blood volume

An initial 10 cc. sample of blood was taken from an antecubital vein without stasis. The mouthpiece and nose-clip were applied and 100 to 130 cc. of carbon monoxide were admitted to the bag. Oxygen was used to wash in the last traces of the carbon monoxide, and was thereafter supplied when needed. An attempt was made to leave as little oxygen in the bag at the end of the rebreathing period as was compatible with comfort in breathing. Rebreathing continued for 15 or 20 minutes, although absorption and mixing were actually complete in less than 15 minutes (10, 23). Before or within a minute after disconnecting the rebreathing system from the patient, a second 10 cc. blood sample was obtained. Samples of gas were then withdrawn from the rebreathing bag for analysis of their carbon monoxide content. The procedure in dogs was very similar, except that smaller amounts of carbon monoxide, 30 to 65 cc., were given. A mask, made of a funnel fitted with a piece of automobile inner tube and sealed around the dog's muzzle with crude petrolatum, was used.

Blood volume was calculated by the formula:

$$\text{B.V.(cc.)} = \frac{[(\text{cc. of stock CO given}) \times (\text{per cent CO in stock gas})] - (\text{cc. residual CO})}{\text{CO of second blood (cc. per cc.)} - \text{CO of first blood (cc. per CC.)}}$$

RESULTS

The data from normal human subjects are presented in Table II, while those from normal and splenectomized dogs are presented in Table III. In the final columns of both tables are the ratios of blood volume by the dye method to blood volume by the monoxide method. Since the error of each method approximates 3 or 4 per cent, ratios falling between 0.95 and 1.05 are not certainly different from unity. In the human experiments (Table II), only 7 of 13 ratios fall within this range, 3 lying above and 4 below. In the dog experiments (Table III), the results are very similar; 8 fall within the range of equality, 6 fall above and 5 below. Occasional ratios differ from unity by an amount much exceeding any possible experimental error. In 2 of the human experiments and in 7 of the dog experiments, the ratios fell below 0.90 or exceeded 1.10. In summary, the 2 methods gave virtually identical results in about half the individual instances, while in the remainder they differed.

TABLE II

Blood volumes of normal human subjects, measured simultaneously by the use of carbon monoxide and by the use of Evans blue dye (T-1824)

Subject	Sex	Age	Weight	Height	Date	Relative cell volume	Total blood volume 1 CO method	Total blood volume 2 Dye method	Ratio, 2:1
		years	*kgm.*	*cm.*	*1942*	*per cent*	*cc.*	*cc.*	
S.J.	F	21	57	163	Sept. 14	38.0	5000	4530	0.91
					Oct. 22	35.2	4835	4600	0.95
M.O.	F	20	47	168	Sept. 21	46.4	3470	3730	1.08
					Oct. 21	39.4	3830	3600	0.94
P.H.	F	40	55	153	Sept. 4	39.3	3750	3740	1.00
					Oct. 7	40.9	3490	3565	1.02
D.H.	F	31	68	178	Sept. 23	40.3	5190	5200	1.00
E.R.	M	23	66	179	Sept. 12	46.8	5800	5310	0.92
					Oct. 9	45.8	5700	5350	0.94
J.W.	M	23	78	188	Sept. 28	46.7	6950	7800	1.12
B.K.	M	30	80	176	Oct. 8	47.5	5475	6330	1.16
D.S.	M	22	63	182	Oct. 14	47.1	6060	6100	1.01
E.H.	M	42	69	175	Sept. 9	47.9	5420	5640	1.04

Sometimes one and sometimes the other method gave the larger value. Partly, at least, as a consequence of this fact, average values for blood volume by the 2 methods, in terms of cc. per kgm. body weight, were almost identical. This is shown in Table IV. All dogs were grouped together for the purpose of calculating the average value, since splenectomized dogs do not appear to differ significantly from normal ones with respect to the distribution of carbon monoxide and of the dye T1824 (Table III).

DISCUSSION

Other observers have compared blood volume measurements by the carbon monoxide method with those obtained by various dye methods (4 to 10). Since the conclusions reached by different workers are not entirely in agreement, either among themselves or with the results presented here, they will be briefly reviewed.

Asmussen (4) stands alone in stating, in a recent publication, that the carbon monoxide method gives consistently larger values than does the Evans blue dye method. This conclusion is not, however, well supported by his own data. In the first place, in nearly half the determinations, the differences between the blood volume figures by the 2 methods do not, in fact, exceed those to be expected from the error of the methods used. Also, using Asmussen's own figures, it may be demonstrated that there is no significant statistical difference between the average of the blood volumes determined by the monoxide method and the average of those determined by

TABLE III

Blood volume of normal and splenectomized dogs, measured simultaneously by the use of carbon monoxide and by the use of Evans blue dye (T-1824)

Dog*	Weight	Date	Relative cell volume	Total blood volume 1 CO method	Total blood volume 2 Dye method	Ratio, 2:1
	kgm.	*1943*	*per cent*	*cc.*	*cc.*	
1	14.6	Jan. 7	57.1	1590	1330	0.84
2	12.7	Jan. 28	45.2	1100	(794)‡	(0.72)
3	8.5	Feb. 9	44.4	965	979	1.01
	8.5	Feb. 24	41.3	890	934	1.05
4	8.5	Feb. 15	32.0	950	800	0.84
5	6.5	Mar. 4	46.6	660	700	1.06
6	9.6	May 10	47.4	960	940	0.98
7	28.1	Jan. 25	49.5	2350	2650	1.13
8		Feb. 17†	40.7	719	754	1.05
9	11.5	Feb. 24†	59.0	1220	1200	0.98
S1	9.3	Mar. 17	31.2	854	837	0.98
	9.5	Mar. 29	29.5	800	912	1.14
S2	6.8	Apr. 15	38.0	560	576	1.03
S3	6.3	Apr. 15	38.4	675	654	0.97
	6.7	Apr. 29†	28.1	579	670	1.16
S4	17.2	May 6	40.8	1315	1395	1.06
	17.3	May 12†	41.3	1405	1590	1.13

* Prefix "S" indicates splenectomized animals.
† Dial anesthesia, carbon monoxide administered by tracheal cannula.
‡ Some hemolysis with possible resultant inaccuracy in dye measurement.

the dye method. The only support for Asmussen's conclusions comes from certain sporadic experiments in which the volumes by the monoxide method greatly exceeded those by the dye method. Asmussen used a large rebreathing chamber (10-liter capacity) for the administration of carbon monoxide, and did not measure or otherwise correct for residual gas. Our own experience with a large rebreathing apparatus indicates that the amount of residual carbon monoxide is most variable, and is often large. If not corrected, this error would therefore make the blood volume by the carbon monoxide method larger than it should be by a variable amount. Neglect of this fact may account for some of Asmussen's aberrant findings. The use of an extrapolated value for dye concentration in some studies but not in others may have been another contributory factor.

Various workers have found larger blood volumes with dye methods than with the carbon monoxide method (5 to 10). Thus Smith, Belt, Arnold, and Carrier (6), using brilliant vital red and carbon monoxide alternately every 1 to 3 days, found the blood volume in man about 30 per cent higher by the dye than by the carbon monoxide method. The average blood volume of normal man by the carbon monoxide method in 20 experiments was 71 ± 5 cc. per kgm. body weight, while in 20 comparable determinations by the dye method it was 93 ± 8 cc. per kgm. body weight. Their methods differed from ours in numerous technical details. For example, only 4 minutes were allowed for mixing time and 10 minutes for the absorption and distribution of carbon monoxide, although 2 to 3 times as much gas as that given in our experiments was administered. McIntosh (10) reported similar results in 10 normal infants, the mean blood volume by the monoxide method being 71 ± 2 cc. per kgm. body weight, that by the dye method 93 ± 10 cc. per kgm. body weight. His technics were similar to those employed by Smith and his associates, except that the determinations were done simultaneously. Bazett and his associates (5) found that the average blood volume measured with Congo red dye exceeded the average volume with the monoxide method by some 8 per cent.

In dogs, Lee, Carrier, and Whipple found a quite similar difference between the results of the carbon monoxide method and of the dye method (7, 8). For example, converting the results of a typical experiment of theirs into terms comparable with our own, in 14 dogs the average blood volume was 87 ± 4 cc. per kgm. of body weight, measured by carbon monoxide, while by the dye method, it was 104 ± 4 cc. per kgm. of body weight. Smith, Arnold, and Whipple (9) obtained similar results, and found that the viviperfusion method gave values for cell mass comparable to the carbon monoxide values rather than to those given by the dye method. More recently, Hahn and his associates (28) found that a new method for cell volume based on the use of radioactive iron in erythrocytes gave values similar to those obtained by the viviperfusion method, and therefore comparable to those given by the carbon monoxide method. The volumes were definitely smaller than those obtained by these same workers with brilliant vital red dye.

TABLE IV

Average whole blood, cell, and plasma volumes by Evans blue dye method and by carbon monoxide method, expressed in terms of cubic centimeters per kilogram body weight

Data derived from Tables I and II

	Blood volume		Cell volume		Plasma volume	
	Dye method	CO method	Dye method	CO method	Dye method	CO method
	cc.	cc.	cc.	cc.	cc.	cc.
Human subjects (9)	80.5± 8.6	80.2± 5.5	35.0	34.9	45.5	45.3
Dogs (16)	95.2±12.4	95.4±12.0	39.8	40.3	55.4	55.1

The results obtained by these investigators are clearly inconsistent with our own observations. Their absolute values by the carbon monoxide method in man are a little lower than ours, while their values for plasma volume by the brilliant vital red method are much higher than those obtained by us with the Evans blue dye, T1824. This discrepancy may possibly be in part ascribed to the difference between the 2 dyes, since their volumes of distribution are only approximately identical (29). Differences in technic of administration may also play a rôle. The use by us of a single 10-minute blood sample in calculating the plasma volume tends to make our values higher than would a 4-minute sample, so this cannot explain the fact that our values are smaller than theirs. Gregerson (30), reviewing

in 1938 the errors encountered in the use of brilliant vital red, concluded that failure to recognize these difficulties renders almost all previous blood volume studies with this technic suspect. Congo red is now known to be an unsatisfactory dye for blood volume estimations (31). Certainly in comparing results obtained with modern technics, using T1824, with older work using brilliant vital red or other dyes, it is reasonable, other things being equal, to attach greater weight to the more recent work.

Although the blood volume values calculated from the apparent volumes of distribution of carbon monoxide and of dye are frequently numerically identical, it does not necessarily follow that this common figure therefore represents the true blood volume. Numerical coincidence in normal subjects does not even prove that the 2 methods are measuring exactly the same physiological compartment, since errors due to one cause with the monoxide method may well chance to be numerically equal to errors due to some quite different cause in the dye method. There is evidence, discussed elsewhere (32, 3), that dye measures a space much larger than the vascular space, owing to loss of dye to lymphatics and elsewhere. Insofar as the carbon monoxide method gives a comparable or higher value for plasma volume, it follows that carbon monoxide also may be distributed over a space larger than the vascular space. There is good reason for believing that there is no appreciable loss of carbon monoxide to myoglobin of muscle (summarized in (3)). The fact that our carbon monoxide and dye measurements bore the same relationship to one another in splenectomized and in normal animals suggests that carbon monoxide is not lost to the spleen; otherwise some systematic difference between measurements in normal and in splenectomized animals might be expected. The possibility exists that carbon monoxide in red cells of the circulating blood may shift into red cells of the bone marrow, resulting in too large a value for blood volume.

In general, it may be concluded that neither method has been shown regularly to measure absolute volume. Their reliability as *relative* measures of blood volume must be assessed by studying their variations under circumstances in which blood volume is known to vary. This problem is dealt with elsewhere (3).

SUMMARY

1. Measurements of blood volume in normal men and dogs by the distribution of T1824 and of carbon monoxide, given simultaneously, were often but not always found to be numerically equivalent.

2. In some subjects, the dye method gave the larger values, in others the carbon monoxide method. *Average* values for blood volume per unit body weight were almost identical by the 2 methods.

3. There is no proof at present that either method regularly measures absolute blood volume.

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SIMULTANEOUS MEASUREMENTS OF THE BLOOD VOLUME IN MAN AND DOG BY MEANS OF EVANS BLUE DYE, T1824, AND BY MEANS OF CARBON MONOXIDE. II. UNDER ABNORMAL CONDITIONS, INCLUDING SECONDARY SHOCK [1]

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The results of simultaneous measurements of the volume of distribution of carbon monoxide and that of Evans blue dye (T1824), in normal man and in normal and splenectomized dogs, have been reported elsewhere (1). It was found that, although measurements of the blood volume by the 2 methods were frequently numerically equivalent, occasionally either carbon monoxide or dye might measure a larger space. On the average, the results with the 2 methods were very nearly the same, in spite of these occasional variations. In the present study, the same method of investigation has been applied to pathological subjects. Studies, both of patients and of dogs, have been carried out. The human subjects consisted of hospitalized patients with various disorders, affecting either the state of the vascular system or the chemical constitution of the body fluids. The dogs were first studied in their normal state, and again after being subjected to various procedures likely to affect the vascular system. These experimental procedures included traumatic shock, salt and water depletion, hemorrhage, adrenal insufficiency, and the injection of epinephrine. The extent to which the normal relationship existing between the results obtained by the 2 methods persisted in pathological states could thus be determined. Furthermore, in the dog experiments, the reliability of these 2 methods as relative measures of blood volume could to some extent be determined. The results could also be compared in this respect with parallel estimations of changes in blood volume calculated from changes in concentration of serum protein and from changes in the fraction of cells in whole blood as determined by the hematocrit.

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METHODS

The methods have for the most part been described elsewhere (1, 2). The details of the technic of simultaneous measurements with dye [4] and with carbon monoxide, of the handling of blood, of hematocrit determinations, and of the general procedure were the same as in the previous study (1). Serum protein concentrations were determined by the macro-Kjeldahl technic (3). The formulae used for calculating plasma volume changes from changes in serum protein concentration and from changes in relative cell volume are as follows:

$$\frac{PV_2}{PV_1} = \frac{SP_1}{SP_2} \qquad (1)$$

and

$$\frac{PV_2}{PV_1} = \frac{(1 - Hkt_2)(Hkt_1)}{(1 - Hkt_1)(Hkt_2)} \qquad (2)$$

where PV_1 = initial plasma volume
PV_2 = subsequent plasma volume
SP_1 = initial serum protein
SP_2 = subsequent serum protein
Hkt_1 = initial fraction of cells in whole blood
Hkt_2 = subsequent fraction of cells in whole blood

Procedue in shock due to ligature

In the shock experiments, a preliminary blood volume study was done with or without the use of "Dial" anesthesia (0.6 cc. per kgm. body weight). Frequently, additional control blood volume studies were done, including one under anesthesia just before the induction of shock, with a tracheal cannula in place for the administration of carbon monoxide. To produce shock, a hind leg was tightly ligated with rubber tubing. The following morning, 8 to 12 hours later, the rubber tubing was removed. Two to

[4] Blue dye T-1824 furnished by the Warner Institute of Therapeutic Research, New York.

3 hours after removal of the tourniquet, when the hind extremity had become edematous and when signs of shock were well developed, the blood volume was again determined. Subsequent blood volume and blood chemical determinations were done in some experiments (Table II). At death, the 2 hind legs were removed and the difference in weight between the edematous and the normal limb determined.

"Dial" anesthesia has a marked effect upon the spleen and upon the distribution of red cells in the circulation (4, 5). Since, however, "Dial" was administered before the control period and its action persisted during subsequent periods, these effects were present at all times, and hence could not have been responsible for the observed changes in blood volume. This conclusion was confirmed in certain experiments in which splenectomized animals under "Dial" anesthesia were used for shock studies. These animals behaved as did other shocked animals with respect to blood volume changes.

Procedure in salt and water depletion experiments

Control studies followed by studies under various conditions of depletion were made as indicated in Table III. One dog (22B), dehydrated by deprivation of water and food, was also depleted of salt by the withdrawal of peritoneal fluid some hours after the intraperitoneal injection of a 5 per cent glucose solution (6). In one normal dog (26A), peritoneal fluid was withdrawn a few hours after the injection of 10 per cent urea dissolved in the 5 per cent glucose solution. This resulted eventually in a large depletion of water, as well as of salt, since the absorbed urea provoked a copious diuresis. Two dogs (25A, 26B) served as controls for this procedure. They received no urea in the 5 per cent glucose solution, so that salt depletion alone resulted. Finally, 2 dogs (25B, 29), initially dehydrated by prolonged withdrawal of food and water, subsequently received repeated intraperitoneal injections of a 5 per cent sodium chloride solution. Certain data from these last 2 experiments have been published elsewhere (11).

Procedure for studies after adrenalectomy

The dog used in this study had been adrenalectomized 2 weeks previous to the time experiments were begun and in the interval had been adequately maintained on salt, cortin, and desoxycorticosterone. Simultaneous measurements were then made both when the animal was adequately maintained on drug therapy, and again after withdrawal of therapy, as indicated in Table IV. In these experiments, as in the hemorrhage experiments, residual carbon monoxide in the lung-bag system was not determined, a constant correction factor being used. Similarly, the mask for administering carbon monoxide was not sealed with petrolatum. Sulfocyanate space was measured by the method of Elkinton and Taffel (7).

Procedure in hemorrhage experiments

A single animal was used. Several control blood volumes were first done. The animal was then bled 200 to 400 cc. daily on 2 or 3 successive days. After an interval of 24 to 72 hours following the last hemorrhage, another blood volume study with both methods was made. Between blood volume studies, the animal was well fed. The usual 12- to 18-hour fast, however, preceded all blood volume measurements. In these studies, residual carbon monoxide in the lung-bag space was not determined. Instead, an arbitrary constant correction was made. Also, in these studies, the mask for administering carbon monoxide was not sealed around the dog's muzzle with petrolatum. Failure to observe this precaution may have resulted in loss of carbon monoxide from the mask in certain of the studies.

Procedure in experiments in which epinephrine was used

To study the vascular effects of epinephrine upon simultaneous carbon monoxide and dye measurements, control blood volume studies were done in the usual way. Immediately after taking the final blood samples for the control study, and with the mask in place so that the animal remained in equilibrium with the carbon monoxide, 1 cc. of 1:1000 epinephrine was given rapidly intravenously. No further injection of dye was made. During the following $2\frac{1}{2}$ to 10 minutes, 1 or 2 blood samples were taken for the simultaneous measurement of carbon monoxide and of dye concentrations and for hematocrit and serum protein studies. Dye measurements are omitted since it was not possible to distinguish quantitatively between the effects on concentration of dye in serum of continuing dye loss and those due to alterations in plasma volume induced by epinephrine.

RESULTS

In patients with various clinical conditions

In Table I are presented the results of 22 studies in 11 patients in whom measurements were made simultaneously with carbon monoxide and T1824. These hospitalized subjects suffered from various diseases, indicated in Table I. With such a variety of diagnoses, and with various stages and varieties of these diseases represented, mean values for the group must be interpreted even more cautiously than were those in a corresponding normal group (1). The ratios of blood volume measurements by the 2 methods are calculated in the final column of Table I. Only 6 of the 22 ratios fall between 0.95 and 1.05, the range of probable equality, and only 9 between 0.90 and 1.10, the extreme range of possible equality (1). Of the remaining 13 ratios, 12 exceed 1.10 while only 1 falls below 0.90. The measurements by the dye method therefore exceed those by the monoxide method in more than half the cases, and give virtually the same results

TABLE I

Simultaneous measurements of blood volume with a carbon monoxide method and with a dye (T1824) method in patients with various diseases

Subject	Sex	Diagnosis	Date	Body weight	Height	Serum protein	Relative cell volume	Blood volume 1 CO method	Blood volume 2 Dye method	Ratio 2:1
			1942	*kgm.*	*cm.*	*grams per cent*	*per cent*	*cc.*	*cc.*	
S.B.	M	Acute nephritis	Apr. 22	56.0	173	6.33	47.0	4380	4960	1.13
		? hydronephrosis	May 27	55.0	173	7.46	51.0	3965	4490	1.13
			July 1	52.4	173	6.53	38.6	3950	3900	0.99
D.A.	F	Mercury poisoning, mild	May 13	55.5	158	7.47	36.1	3930	4730	1.20
			May 25	50.0	158	7.11	38.6	3675	4110	1.12
G.H.	M	Addison's disease treated	July 6	57.0		5.62	32.2	5185	5375	1.04
			July 8	56.1		6.11	29.5	4690	5118	1.09
			July 13	57.1		5.75	28.9	5930	5438	0.92
S.M.	F	Addison's disease treated	Oct. 16	63.0	156	7.12	38.5	3900	3300	0.85
			Oct. 30	63.0	156	5.69	34.2	3870	3585	0.93
H.S.	F	Hyperthyroidism, before and	Apr. 10	48.0	158	7.02	46.0	3475	3980	1.15
		after operation	Apr. 29	48.0	158	7.32	32.6	3605	3450	0.96
F.C.	F	Hyperthyroidism, before and	June 8	88.0		6.87	49.0	5350	6170	1.15
		after operation	June 25	88.0		6.43	48.3	5000	6140	1.23
M.M.	F	Hyperthyroidism, before and	June 10	59.0	155	6.72	45.7	3330	3820	1.15
		after operation	June 24	59.0	155	6.28	38.8	3500	3490	1.00
H.R.	F	Portal cirrhosis	May 20	50.4		6.42	54.3	3910	5690	1.46
N.C.	M	Portal cirrhosis	May 1	69.7		5.05	34.4	6340	6560	1.04
B.L.	M	Cardiac decompensation at	Apr. 15	62.0		4.88	39.2	5150	5940	1.15
		different stages of recovery	Apr. 27	54.4		5.61	37.9	5245	5340	1.02
			May 8	57.0		5.41	35.5	4800	5970	1.24
H.E.	M	Lymphogranuloma inguinale	Apr. 24	69.1		7.98	47.9	4080	5780	1.42

in most of the remaining ones. Correlation of the ratios with clinical diagnosis cannot be attempted because there are too few cases of any one type.

In shock due to ligature in normal and splenectomized dogs

The results of these experiments are presented in Table II. Nine dogs were used in these experiments, 5 of which were normal and 4 splenectomized. Twenty-five simultaneous determinations of the blood volume were done. As indicated by both methods, blood volume regularly decreased following the establishment of shock. In 1 instance only (experiment 1), the dye method remained unchanged, and even here the monoxide method recorded a decline. The magnitude of this decrease was greater by the monoxide than by the dye method in about half the experiments, and was of about the same degree in the remainder. This is indicated by the behavior of the ratios between the 2 measurements, recorded in the final column. During shock, dye T1824 frequently measures a much larger volume than does carbon monoxide, the magnitude of some of the ratios greatly exceeding those ever found in normal circumstances. The differences between the behavior of the measurements in different experiments may be related to the fact that the intensity and duration of shock varied considerably from experiment to experiment.

The decline in blood volume occurred mainly at the expense of the plasma fraction. This was to be expected from the nature of the experimental procedure, which causes a transudation of fluid containing plasma, but few erythrocytes, into the tissues of the ligated limb immediately on removal of the ligature (8). Little change in total cell volume should therefore be recorded by a reliable measurement of blood volume; a small decrease only might be expected as the result of removal of blood for analytical purposes. The total cell volumes measured by each of the 2 methods are presented in Table II. The expected result—no essential change or a slight decrease—was found in about half the experiments. In the remaining experiments, the dye method recorded an increase 3 times and a sharp

decrease only once, while the monoxide method recorded an increase once and a striking decrease 3 times. These results suggest that, on occasion, both methods are quite unreliable, but they tend to err in opposite directions. The monoxide method sometimes may give lower than usual values for the blood volume in this type of shock, while the dye method may give values higher than usual.

In dehydrated animals with and without salt depletion

In Table III are presented the results in 6 experiments in which water or salt content of the body had been altered. Acute salt depletion of sufficient severity will induce cardiovascular collapse, secondary shock, and death (9). This procedure was carried out 5 times in 4 experiments (22B, 25A, 26A, 26B). The monoxide method indicated a fall in blood volume in every case in which it was carried out, while the dye method indicated a fall only 2 times out of 5. There was, therefore, a dissociation between the results by the 2 methods in the remaining 3 instances, and the ratio between them rose. Simple water deprivation results in loss of body water from all compartments (10), and might be expected to reduce blood volume if it altered it at all. In the 3 experiments with prolonged water deprivation (22B, 25B, and 29), blood volume as measured by both methods did consistently decline. Water deprivation combined with injection of hypertonic saline, on the other hand, tends to maintain or expand the volume of

TABLE II

Simultaneous measurements of blood volume of dogs with a carbon monoxide method and with a dye (T1824) method, before and during secondary shock, induced by release of a ligature constricting the thigh

Dog†	Date	Condition	Gain in weight of leg	Body weight	Serum protein	Relative cell volume	Cell volume		Blood volume		Ratio 2:1
							CO method	Dye method	1 CO method	2 Dye method	
	1943		*grams*	*kgm.*	*grams per cent*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	
1	Jan. 14 (a.m.)‡	Control		8.0	5.80	61.5*	294	371	478	613	1.28
	Jan. 14 (p.m.)‡	Shock			5.51	61.2	217	378	355	618	1.74
2	Jan. 25	Control		28.1	7.79	49.5	1163	1312	2350	2650	1.13
	Jan. 26‡	Shock	670		7.52	65.2	1180	1258	1810	1930	1.07
3	Feb. 1	Control		8.3	4.64	35.0	412	333	1175	950	0.81
	Feb. 2‡	Shock	130			52.4	372	392	710	748	1.05
4	Feb. 10	Control		10.0	5.12	47.7	604		1265	1145	0.91
	Feb. 11‡	Shock				65.4	374		572		
	Feb. 12‡	Shock			5.62	57.1	483		845		
5	March 1‡	Control		8.8		56.7	595	544	1050	960	0.91
	March 2 (a.m.)‡	Shock				70.9	428	490	605	692	1.14
	March 2 (p.m.)‡	Shock	270			70.9	321	512	453	722	1.59
S100	March 17	Control		9.3		31.2	267	261	854	837	0.98
	March 29	Control		9.5		29.5	236	269	800	912	1.14
	April 7‡	Control			5.65	33.1*		278		840	
	April 8‡	Shock	420		5.23	46.1*	321	352	696	764	1.10
S101	April 15	Control		6.8	5.01	38.0	213	219	560	576	1.03
	April 22‡	Shock	50	7.0	5.31	43.8	187	200	427	457	1.07
S102	April 15	Control		6.3	4.97	38.4	259	251	675	654	0.97
	April 29‡	Control		6.7	5.35	28.1	163	188	579	670	1.16
	April 30 (a.m.)‡	Shock			5.72	26.9	144	129	534	479	0.90
	April 30 (p.m.)‡	Shock			5.48	24.0	115	137	480	572	1.19
	May 1 (a.m.)‡	Shock			5.80	22.5		112		500	
	May 1 (p.m.)‡	Shock	100		5.96	20.0	110		505		
S103	May 6	Control		17.2	5.95	40.8	536	569	1315	1395	1.06
	May 12‡	Control			5.83	41.3	580	657	1405	1590	1.13
	May 13 (a.m.)‡	Shock			5.97	50.0	503	558	1005	1115	1.11
	May 13 (p.m.)‡	Shock			5.92	50.0	448	756	896	1510	1.69
	May 14 (a.m.)‡	Shock			5.77	44.3	382	558	863	1260	1.46
	May 14 (p.m.)‡	Shock	225			39.3					

* Venous instead of arterial blood used.
† Letter "S" refers to splenectomized dogs.
‡ Anesthetized with Dial and tracheal cannula in place.

the extracellular fluid at the expense of the intracellular (11). Blood volume might therefore be expected to increase, at least for a time. In the 2 experiments bearing on this point (experiments 25B and 29), the monoxide method indicated an increase in one and no change in the other. In the single experiment in which dye measurements were made, there was a moderate decrease of blood volume, not indicated by the simultaneous monoxide method.

Taking the results of these experiments all together, it appears that, in most instances, both methods tend to confirm one another, and indicate changes in the direction to be predicted on physiological grounds. The single important exception is the failure of the dye method to record any decline in blood volume in certain instances of shock due to acute salt depletion (22B, 25A), in which there is much reason to believe that some decline occurred. Because changes in plasma salt concentration such as were produced in these experiments radically affect relative cell volume without necessarily altering the blood volume, calculations of total cell mass by the 2 methods are of little value in assessing the relative soundness of the 2 methods, and so have been omitted.

In the adrenalectomized animal

Simultaneous measurements after adrenalectomy (Table IV) are characterized for the most part by failure of the 2 methods to agree, the ratios varying from 0.75 to 1.54 without evident relation to the clinical state. In the studies of May 18 and of July 16, when treatment of adrenal cortical insufficiency was definitely inadequate, as indicated by the contraction of the sulfocyanate space, both methods indicated lower

TABLE III

Simultaneous measurements of blood volume by a carbon monoxide method and by a dye (T1824) method, in dogs subjected to water and salt depletion and in dogs receiving injections of hypertonic saline

Dog‡	Date	Procedure	Body weight	Serum protein	Relative cell volume	Blood volume 1 CO method	Blood volume 2 Dye method	Ratio 2:1
	1943		*kgm.*	*grams per cent*	*per cent*	*cc.*	*cc.*	
22B	Jan. 7 (a.m.)	Normal control	14.6	5.08*	57.1*	1590	1330	0.89
	Jan. 7 (p.m.)	After acute salt depletion	14.5	6.17*	61.3*		1405	
	Jan. 9	Continued water deprivation	13.2	6.42*	56.8*	1200	1350	1.12
	Jan. 11	Continued water deprivation	12.4	6.72*	59.9*	1390	1150	0.83
	Jan. 12	Continued water deprivation, further salt depletion	12.2	7.09*	54.6*	1250	1122	0.90
	Jan. 15	Continued water deprivation	11.2	6.57*	51.8*	1120	860	0.77
25A	Feb. 9 (a.m.)	Normal control	8.5	6.15	44.4	965	979	1.01
	Feb. 9 (p.m.)	After acute salt depletion	8.4	7.08	49.7	842	1020	1.21
	Feb. 10	Water by mouth without salt restoration	8.2	6.03	41.8	792	928	1.17
25B	Feb. 24	Normal control	8.5	5.86	41.3	890	934	1.05
	March 4	Water deprivation since Feb. 24	7.0	6.83	47.4	700		
	March 6	Hypertonic saline; water deprivation	6.6	6.08	42.8	633		
	March 8	Hypertonic saline; water deprivation	6.3	5.44	31.0	873		
26A	Feb. 5 (a.m.)	Normal control	9.3	4.59	35.0	†	921	
	Feb. 5 (p.m.)	After acute salt depletion	8.8	5.78	44.4	770	746	0.97
	Feb. 6	After salt and water depletion	8.0	5.19	32.3	746	776	1.04
26B	Feb. 15 (a.m.)	Normal control	8.5	4.74	32.0	950	800	0.84
	Feb. 15 (p.m.)	After acute salt depletion	8.1	5.84	37.5	524	670	1.28
29	Feb. 24	Normal control	7.9	5.67	45.4	884	828	0.94
	March 4	Water deprivation since Feb. 24	6.5	5.75	46.6	660	700	1.06
	March 6	Hypertonic saline; water deprivation	6.1	5.43	38.7	685	619	0.90
	March 8	Hypertonic saline; water deprivation	5.7	5.31	34.8	611	617	1.01
	March 10	Hypertonic saline; water deprivation	5.3	5.04	25.2		538	

* Venous instead of arterial sample used.
† See control studies of same animal under 26B, Feb. 15.
‡ Numbers refer to individual dogs, letters to individual experiments on the same dog.

TABLE IV

Simultaneous measurements of blood volume by a carbon monoxide and by a dye (T1824) method of blood volume in an adrenalectomized dog, treated with varying amounts of DOCA and of cortical extract

Date	Body weight	Status of treatment*	SCN space	Serum protein	Relative cell volume, venous	Blood volume 1 CO method	Blood volume 2 Dye method	Ratio 2:1
1942	*kgm.*		*cc.*	*grams per cent*	*per cent*	*cc.*	*cc.*	
May 18	9.1	Adequate treatment with a little cortin	2560	7.54	51.4	567	632	1.11
28	9.8	Well treated with cortin and DOCA	2960	5.88	31.0	945	713	0.75
June 3	9.6	Well treated with cortin and DOCA		5.53	38.5	977	762	0.78
11 (a.m.)	10.0	Well treated with cortin alone	3120	5.56	38.2	824		
11 (p.m.)	10.0	Well treated with cortin alone			32.0		794	
18	10.7	Well treated with DOCA alone	3270	5.94	41.2	705	847	1.20
July 10	9.4	Just maintained on cortin and DOCA	2900	5.73	50.4	724	1112	1.54
16	9.0	No cortin or DOCA since July 10	2010	6.83	58.6	734	604	0.82
24	9.9	Well treated with DOCA alone	3160	5.26	29.5	636	750	1.18

* DOCA means desoxycorticosterone acetate.

blood volumes than they did during studies in which treatment was more adequate. The decline was mainly at the expense of the plasma volume. Measurements of both methods therefore indicated correctly the decline in the plasma volume which almost certainly accompanies inadequate treatment (12), being much lowered when treatment was discontinued or insufficient. The technical difficulties in using carbon monoxide, mentioned in experiments with hemorrhage, are present in this study as well, and may account for some of the variability in the ratios.

In hemorrhage experiments

In Table V are presented the pertinent data from experiments done following repeated hemorrhages. Internal evidence, in the form of agreement between most successive determinations and between carbon monoxide and T1824 measurements, suggests that, in spite of the occasional

TABLE V

Simultaneous measurements of blood volume by a carbon monoxide method and by a dye (T1824) method in a dog subjected to repeated hemorrhages

Date	Body weight	Amount of blood lost since preceding study	Relative cell volume, venous	Cell volume CO method	Cell volume Dye method	Blood volume 1 CO method	Blood volume 2 Dye method	Ratio 2:1
1942	*kgm.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	
May 8	21.0	0	36.0	732	887	2030	2460	1.21
14	20.6	0	36.9	746	844	2020	2285	1.13
29	20.7	0	46.4	1000	1110	2150	2395	1.12
June 12	21.5	0	43.4	990	929	2280	2140	0.94
17	21.2	600	38.0	831	791	2185	2080	0.95
22	22.2	500	35.2	795	722	2260	2050	0.91
26	20.7	900	26.1	625	495	2395	1895	0.79
29	20.9	300	29.0	693	591	2385	2035	0.85
July 3		960	24.8	567	753	2285	3035	1.33
9	20.4	740	27.3	585	486	2142	1780	0.83
13	19.9	1285	18.9	487		2580		
17		1115	23.0	556	437	2415	1900	0.79
22	18.4	1335	15.1	420	259	2780	1715	0.62
27	19.4	0	23.2	561	483	2420	2080	0.86
Aug. 3	20.9	0	39.4	955	2230	2420	5660	2.34
25	20.5	0	55.2	1223	2520	2215	3550	1.60

technical difficulties (see section above on Methods), most of these blood volume determinations are not unsatisfactory. Excluding the 2 final figures for blood volume by the dye method, which are manifestly incorrect, a mean blood volume of 2310 cc. is obtained by the carbon monoxide method and of 2142 cc. by the dye method, a reasonable correspondence. Of interest are certain determinations which vary widely from the above means and from each other. In one instance, the blood volume determined with carbon monoxide was 2730 cc.; that by the dye, done at the same time, was 1715 cc. In another instance, the dye gave a volume of 5660 cc., a ridiculously high figure. We have no reason for believing that these aberrant values actually represent blood volume changes, nor, in the case of dye measurements, that they are due to faulty technic, because usually, in this animal and in normal animals (1), we obtained reasonable values at other times, using the same technic. In the case of the carbon monoxide measurements, since residual carbon monoxide was not measured, the high values obtained by this method could result from insufficient correction for this residuum. A leak in the carbon monoxide administering system, due to failure to seal the mask with petrolatum, may also well have played a rôle. In the case of the dye, very high and very low values can be explained only by unusual distribution or disposition of the dye. The dye did not disappear with unusual rapidity in experiments with either very low or very high plasma volumes. Had extrapolated rather than 10-minute values been used in calculating blood volume by the dye method, these abnormal values for blood volume would therefore still have persisted.

Simultaneous measurements after intravenous epinephrine

As indicated under the section on methods, technical difficulties prevent a comparison of carbon monoxide and dye measurements before and immediately after giving epinephrine. In Table VI are summarized experiments showing the effect of epinephrine. In the majority, both of normal and of splenectomized animals, the carbon monoxide method indicates that no change of blood volume was produced by epinephrine.

TABLE VI

Measurements of blood volume in normal and in splenectomized dogs before and after the intravenous injection of epinephrine, using the carbon monoxide method

Dog*	Date	Time after epinephrine	Serum protein concentration	Relative cell volume, arterial	Blood volume, CO method
	1943	*minutes*	*grams per cent*	*per cent*	*cc.*
2	Jan. 25	0		49.5	2350
		2		52.3	2220
3	Feb. 1	0	4.64	35.0	1175
		3	4.47	39.1	1173
6	Feb. 17	0		40.7	719
		2		39.8	726
7	Feb. 24	0		59.0	1220
		3.5		68.0	1183
5	Mar. 1	0		57.6	1050
		5		62.0	859
S100	Mar. 17	0		30.1	854
		2.8		30.7	836
		10.3		30.0	858
S100	Mar. 29	0		29.5	800
		3		30.2	848
		7.5		29.4	973
S101	Apr. 15	0	5.01	38.0	560
		3	4.84	34.0	618
S102	Apr. 15	0	4.97	38.4	675
		6.5	4.84	34.2	665
S102	Apr. 29	0	5.35	28.1	579
		4.5	5.60	29.7	560
S103	May 6	0	5.95	40.8	1315
		5	5.71	40.1	1460
S103	May 12	0	5.83	41.3	1405
		4	5.79	43.2	1248

* Numbers are the same as those of Table II, "S" refers to splenectomized dogs.

At times, however, there was a small apparent increase, and at other times, an apparent decrease. Whether these apparent changes represent true changes is questionable, since they may simply result from faulty sampling of the average relative cell volume due to dynamic changes caused by epinephrine.

Comparison of the changes in plasma volume, as determined by carbon monoxide and by T1824, with those calculated from relative cell volume and from serum protein changes

In Figures 1, 2A, 2B, 2C, and 2D, are 5 correlation charts based on data in the preceding tables. Plasma volume changes by dye method and by carbon monoxide method have been calculated from the blood volume figures, and expressed in terms of percentage of initial values. Changes in plasma volume for the same experiment have been calculated from changes in concentration of serum protein and of relative cell volume, accord-

ing to the procedures outlined under Methods. If the 2 methods compared give the same results, points will fall along the diagonal axis; conversely, the distance of points from the axis measures the discrepancy between the results by the 2 methods. Points falling in the upper left or lower right-hand quadrants indicate an inverse correlation.

Plasma volume changes by the monoxide and dye methods are fairly well correlated in a qualitative sense (Figure 1). There are 3 points lying on the abscissa, 2 of them corresponding to those experiments with acute salt depletion in which the monoxide indicated a decline in blood volume but the dye did not. With these exceptions, both methods regularly indicated a change in the same direction. Quantitatively, the correlation is not satisfactory, since points are widely scattered on both sides of the diagonal line. More points are above than below the diagonal, indicating that in a majority of cases the dye method indicated less of a change than did the monoxide method.

Changes in plasma volume as measured by change in plasma protein concentration are compared with simultaneous measurements of change in plasma volume by the dye and monoxide methods in Figure 2A and 2B. Although there is some correlation, the large number of points in the upper left-hand quadrant corresponds to experiments in which the serum protein method indicates an increased plasma volume while the other methods indicate a decreased volume. Since these all occurred in experiments in which a decrease might be expected on physiological grounds, the validity of the serum protein method in these cases must be doubted.

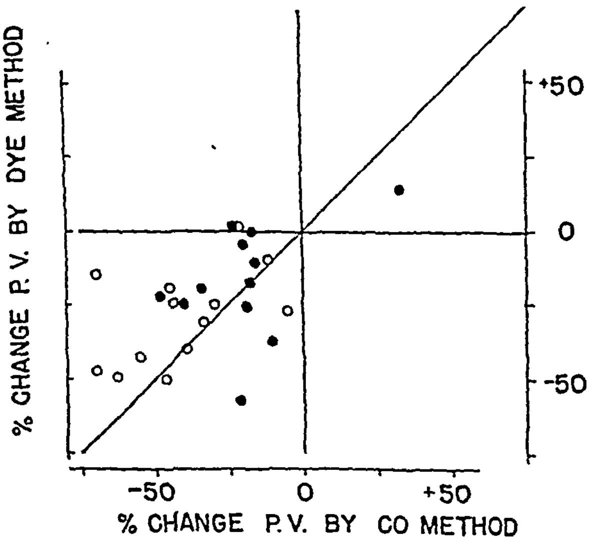

FIG. 1. RELATIVE PLASMA VOLUME. INDIVIDUAL SIMULTANEOUS MEASUREMENTS OF CHANGE IN PLASMA VOLUME BY THE DYE METHOD AND BY THE CARBON MONOXIDE METHOD

Solid circles indicate experiments of Table III; *open* circles all other experiments.

Even more marked is the frequency with which the correlation is inverted between changes measured by the hematocrit method and those measured by the dye and monoxide methods, Figure 2C and 2D. Some of the aberrant points in the upper left-hand corner are derived from experiments with changes in salt concentration of body fluids, which might be expected to affect the validity of the hematocrit method, but others are derived from simple shock experiments in which no such concentration changes occurred.

DISCUSSION

The conclusion was reached in a previous paper (1) that *neither* the dye method *nor* the carbon monoxide method regularly measures absolute blood volume in normal subjects. The experience with pathological subjects tends to confirm and extend this conclusion. In pathological as in normal states, either method may on occasion give the larger value, and it is not possible to assert that either one consistently measures absolute blood volume. There is more tendency in pathological than in normal subjects for the dye measurement to exceed that by monoxide. This tendency toward a larger value for the blood volume in many of the patients may be associated with their particular disease, or it may be due to some non-specific factor common to this pathological group as a whole. For example, the patients had all been confined to bed for some days prior to the determination of blood volume, while the normal subjects had previously been ambulatory. No such explanation seems possible of the frequent high values given by the dye methods in shocked animals. It is possible, however, that the high values in shock resulted from the accelerated rate with which dye escapes from the vascular space in this condition. The valid-

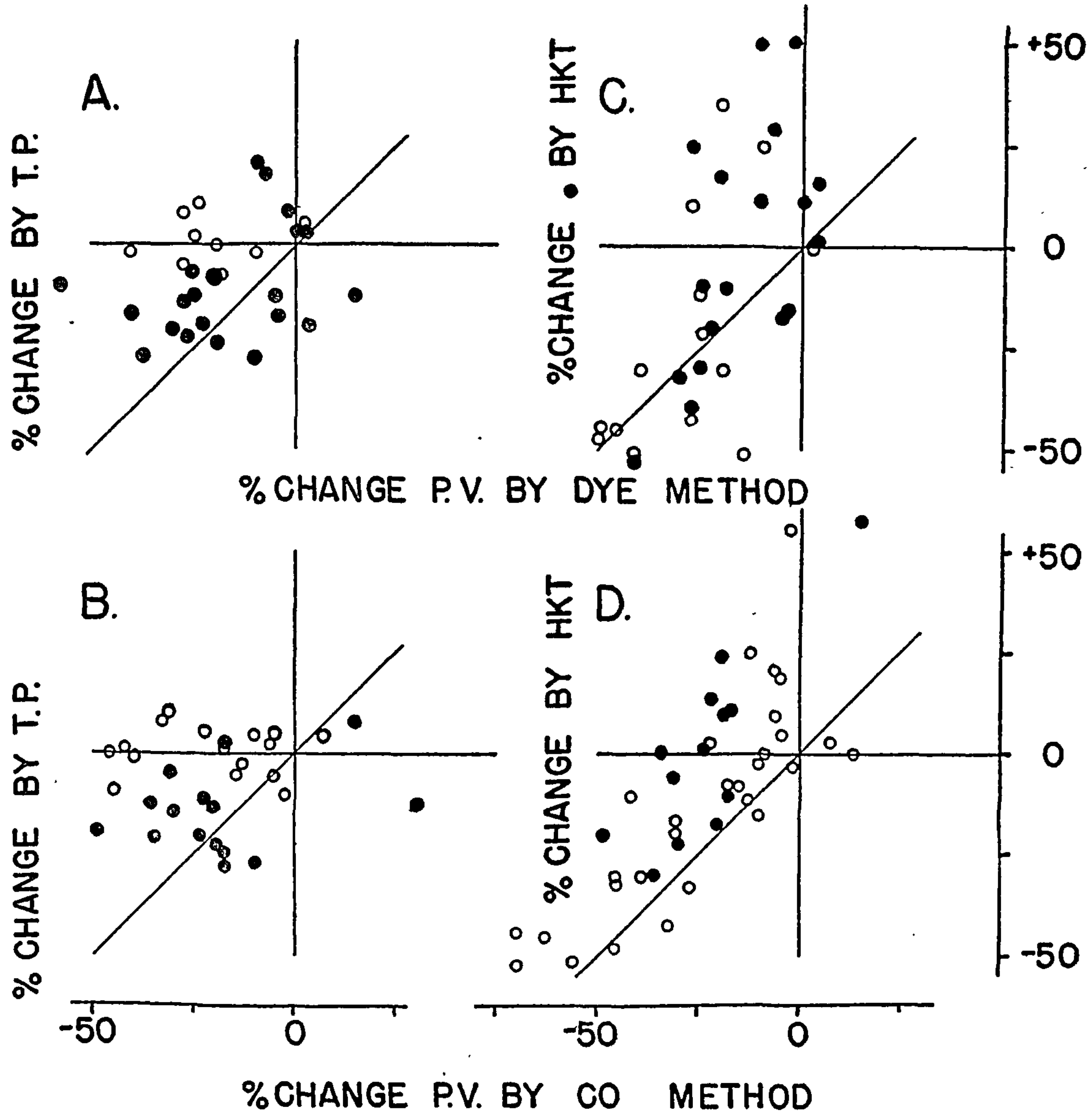

FIG. 2. RELATIVE PLASMA VOLUME. INDIVIDUAL SIMULTANEOUS MEASUREMENTS OF CHANGE IN PLASMA VOLUME BY 4 DIFFERENT METHODS

In A and B, relative plasma volumes calculated from change in total protein (T.P.) concentration in serum are compared with relative plasma volumes measured by the dye method and by the carbon monoxide method, respectively. In C and D, relative plasma volumes calculated from change in the fraction of cells in whole blood (Hkt) are compared with relative plasma volume measured by the dye method and by the carbon monoxide method, respectively. Solid circles indicate experiments of Table III; open circles all other experiments.

ity of the dye method in shock will be discussed more fully later.

On the whole, it seems probable that both methods tend to exaggerate the true blood volume. In previous papers (1, 2), it has been shown that most of the technical errors in the measurement of blood volume by the monoxide method tend to result in values exceeding the actual volume. Some of the reasons which are commonly supposed to support this conclusion are not, however, entirely valid. Thus, it has been suggested that carbon monoxide measures a space larger than the vascular space because some carbon monoxide leaves the intravascular space and combines with the myoglobin of muscle and with the blood pigments in reservoirs such as the spleen and bone marrow. That the loss of carbon monoxide to myoglobin is not responsible for an appreciable error is supported by the finding, repeatedly confirmed by ourselves and by others (13, 14), that the concentration of carbon monoxide in the blood is quite constant

a few minutes after the inhalation of the gas. Even strenuous exercise, which greatly increases the flow of blood through the muscles and so might be expected to facilitate a loss of carbon monoxide to them, does not appreciably affect the carbon monoxide concentration of the blood (13, 14). The slight drop in concentration of carbon monoxide in the blood, observed by Asmussen within the first few minutes of giving carbon monoxide and attributed by him to loss of carbon monoxide to the pigment stores, can just as well be explained by the process of distribution within the vascular space.

There is also much evidence that carbon monoxide is not actively taken up by the red blood cells of the spleen (15). In animals, direct analysis of the spleen showed that carbon monoxide tended to be excluded unless the blood was more than 20 per cent saturated, a saturation rarely attained with the amounts of carbon monoxide used by us or by others in blood volume measurements. Even with high blood concentrations, it took several hours for the same concentration to be reached in the spleen. Our own demonstration that carbon monoxide measures the same volume in splenectomized as in normal dogs likewise indicates that carbon monoxide is not lost to the spleen.

A loss of carbon monoxide to the pigment stores, particularly to those of the bone marrow, is not excluded by our work. Such a loss was in fact postulated by Whipple and his associates (16), Barcroft and his associates (17), and others. In any event, any losses due to escape of carbon monoxide from the intravascular space would tend to produce misleadingly high results. In so far as dye volumes equal or exceed those of carbon monoxide, the dye method must therefore also give too high a value. It does not seem probable, however, that the loss of carbon monoxide to the bone marrow is very great, for the considerations relating to the fixation of carbon monoxide by myohematin are also valid here (13, 14).

There are various reasons for believing that the dye method also regularly gives excessive values for the plasma volume. The validity of this method depends on the assumption that either a negligible amount of dye is lost during mixing time, or that, if the initial concentration is derived by extrapolation from the curve representing the rate of dye disappearance, the dye leaves the circulation at a uniform rate at all times after its injection. Both of these assumptions are open to serious question. Since the dyes used in this method are attached to the protein of plasma (18, 19), it is probably this attachment which prevents the rapid loss of dye from the vascular space. It is therefore possible that some loss of free dye from the circulation may take place immediately after injection, before attachment has had time to develop. The quantitative importance of this factor is unknown, but in so far as it occurs, plasma volume measurements will be exaggerated. More definite is the rapid loss of dye into the lymphatic system (20, 21, 22), which necessarily means that the apparent volume of distribution of the dye, calculated by the usual extrapolation method, must include some of the lymphatic space along with the intravascular space. There is also evidence that the liver may act as a temporary repository for the dye (23, 24, 25). It is not known whether the appearance of the dye T1824 in the tubular epithelium of the kidney (26) occurs rapidly enough to affect the early distribution of the dye, or whether the decolorization of the dye with time occurs *in vivo* as it does *in vitro* (27), but these factors, if present, would further accentuate the exaggeration of the plasma volume by the dye method.

Whipple and his associates (28) still maintain that plasma and cells are not evenly distributed throughout the circulation. The evidence cited in favor of this contention is, however, open to other interpretation. They originally advanced the hypothesis of an uneven distribution in order to explain the smaller values for blood volume obtained with the monoxide method when compared with those obtained with the brilliant vital red dye method. However, upon observing that some dye was lost to the lymphatics, Smith (20) conceded that this might account in part for the greater space measured by the dye than by carbon monoxide. Waterfield (29) has contributed evidence to support this view in the form of experiments in which it was shown that increases in leg volume corresponded to blood volume changes which occurred in changing from the recumbent to the standing position. The

recent study by Stead and Ebert (30), supporting the view that relative cell volume measurements by hematocrit do not reflect the relationship of cells to plasma throughout the body, depends for the validity of its argument on the unwarranted assumption that the dye method measures actual plasma volume. There are, on the other hand, studies such as those of Steinmann (31), in which exactly the same hemoglobin concentration was found in blood taken simultaneously from ear, finger, toe, arm vein, and mesenteric veins; in normal circulatory states the concentration did not vary by more than one part in a hundred.

Our own observations indicate that relative cell volumes of arterial blood and of venous blood are identical except under conditions of profound shock. In shock, it is certainly true that the distribution of cells throughout the vascular system may be uneven, and this factor must be taken into account in comparing dye and monoxide measurements under these conditions. If the measured relative cell volume is higher than the true average relative cell volume, blood volume by the dye method will be too high and that by the monoxide method too low; conversely, if the measured value is less than the average, the distortion by each method will be reversed. In our shock experiments, arterial blood was used; if there was any difference between the relative cell volume in arterial and venous blood, it was always higher in the latter. In other words, if arterial blood were not a fair sample of average blood, it differed by having less than the average proportion of cells; this is reasonable also from what is known of the distribution of blood in shock. This error in sampling would therefore tend to make dye measurements too low and monoxide measurements too high; it cannot therefore be responsible for the fact that dye measurements in shock usually *exceed* those by monoxide.

In our bleeding experiments, the total cell mass, as determined by the carbon monoxide method, bears an approximately linear relationship to the relative cell volume as measured by the hematocrit. Just such a relationship was observed by Hahn and Bale (32) when radioactive cells were used to measure total cell mass. With the exception of certain of the aberrant studies previously alluded to, the cell mass as determined by the dye, T1824, likewise bears a linear relationship to the hematocrit. Hahn, using brilliant vital red and a modification of the procedure used by Whipple, obtained no such relationship (33). This suggests that brilliant vital red as used by these investigators does not measure a space equivalent to that measured by T1824 with the Gibson and Evelyn technic. The correlation of changes in cell mass, as measured by both the carbon monoxide and dye technics, with changes in relative cell volume lends support to the idea that the ratio of cells to plasma is normally the same throughout the circulation.

Certain observations in the course of our experiments indicate that repeated use of the blue dye may lead to anomalous results. In the experiment with repeated bleedings (Table V), dye injections were given many times. Ultimately, sporadic increases of plasma volume as measured by the dye method appeared, much too large to indicate real changes in plasma volume. Lindhard (34) has observed successively larger values for blood volume with prolonged repeated use of the dye, vital red. Thompson and his associates (35), on the other hand, have reported smaller apparent plasma volumes with repeated injections of dye, an observation contrary to that of Lindhard. The explanation of these curious results is at present obscure.

There is much evidence that both methods, although of doubtful value in the measurement of absolute blood volume, may be usefully applied to the detection of changes in blood and plasma volume. The correlation in Figure 1A indicates that reductions in plasma volume are recorded with considerable regularity by both methods, although the extent of the recorded change is not usually the same with the 2 methods. There is a tendency for the dye method to indicate less of a reduction than does the monoxide method. This is especially true under conditions of shock, and may, in a few instances, lead to recording of a reduction by the monoxide but not by the dye method. The increased rate of dye loss in shock (36) undoubtedly tends to exaggerate the plasma volume by the dye method in shock as compared with the normal status. It seems reasonable, therefore, to doubt

the dye rather than the monoxide method under these circumstances.

It is not just, however, to assign all the quantitative change by the 2 methods to vagaries in the dye method alone. In quite a number of cases, the dye method indicates more of a reduction than does the monoxide, a difference which obviously cannot be explained by abnormally rapid loss of dye. There is really no reason to believe that the monoxide method measures relative plasma volume more exactly than does the dye. It is fairly clear that both methods are fairly reliable qualitative, but not quantitative, measures of relative plasma volume. In this respect, they are clearly superior to methods based on changes in serum protein concentration or on changes in relative cell volume. Indeed, although these older methods based on protein and hematocrit measurements are still widely employed in clinical and experimental work (37), they may frequently be seriously misleading, even in a qualitative sense. Failure of the dye method to indicate a reduction of plasma volume in shock does not prove that such a decrease has not taken place. On the other hand, demonstration of a reduction in plasma volume in shock by either dye or monoxide is good evidence that a change actually did take place in the direction indicated.

CONCLUSIONS

1. The relationship between simultaneous measurements of blood volume with the dye, T1824, and with carbon monoxide is more variable in abnormal than in normal subjects.
2. In some abnormal subjects, the dye method tends to give considerably higher values than does the monoxide method. This is especially apt to occur in dogs in secondary shock.
3. It is probable that both methods tend to give absolute values which exceed the true blood volume.
4. Both methods consistently indicate correctly the direction of change in plasma volume. They are therefore useful relative measures of plasma volume, at least in a qualitative sense.
5. The only exception occurs in some cases of traumatic shock, in which the dye method may fail to record a decline in plasma volume.
6. On the other hand, an indication by the dye method that there has been a decline of plasma volume in shock almost certainly means that such a decline has taken place. The dye method can therefore be used in the study of plasma volume in shock, provided these limitations be taken into account in interpreting the results.
7. Dye and monoxide methods both indicate changes in plasma volume more reliably than do changes in serum protein concentration or in relative cell volume, which may, in fact, wrongly indicate even the direction of change.

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EFFECT OF BLOOD SERUM FROM PATIENTS WITH MYASTHENIA GRAVIS ON THE SYNTHESIS OF ACETYLCHOLINE *IN VITRO*[1]

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INTRODUCTION

Walker's observation (1) that neostigmine,[2] a choline esterase inhibitor, benefited patients with myasthenia gravis, suggested that the acetylcholine metabolism was disturbed in such patients. Assuming that this conception has validity, the dominant possibilities concerning myasthenia gravis are: (*a*) excessive destruction of acetylcholine, due to unusually large amounts of choline esterase; (*b*) defects in the ability of muscle to respond to acetylcholine; and (*c*) defects in the synthesis or release of acetylcholine.

The choline esterase level of the serum is not unusual in patients with myasthenia gravis, as was shown by Milhorat (2). Lanari (3), and Harvey and collaborators (4 to 6) demonstrated that the skeletal muscle of patients with myasthenia gravis is capable of reacting to acetylcholine and, indeed, is especially sensitive. Harvey and Lilienthal (5) suggested that this increased sensitivity represented a change in muscle threshold because of its exposure to diminishing amounts of acetylcholine.

The most plausible hypothesis is that there exists a fundamental defect in the synthesis of acetylcholine in patients with myasthenia gravis. Dr. Otto Loewi suggested that such synthesis be investigated.[3]

MATERIAL

The sera of 5 healthy subjects and 59 patients with diseases other than myasthenia gravis were used as controls. Summaries of the clinical state of 9 patients with myasthenia gravis are presented in Table I. In the ninth patient (N), the diagnosis of myasthenia gravis, although probable, was difficult to establish.

EFFECT OF HUMAN SERUM ON THE SYNTHESIS OF ACETYLCHOLINE

Principles and outline of method. The method of Quastel, Tennenbaum, and Wheatley (7) was modified for use in this demonstration. The bases of the method utilized are: (*a*) uniform samples of nerve tissue under standard environmental conditions will synthesize acetylcholine at a fixed rate; (*b*) the amount of acetylcholine thus synthesized may be biologically assayed by measuring its effect in inducing contraction in a properly sensitized muscle. The relative ability of 2 media to influence the synthesis of acetylcholine can thus be measured. In this series of experiments, the nerve tissue was frog brain. The indicator of acetylcholine content was the rectus abdominis muscle of the frog. The effect on acetylcholine synthesis of sera from patients with myasthenia gravis was compared with the effect on acetylcholine synthesis of control sera.

Collection and preparation of samples. The frog brain[4] medium was prepared by mincing with scissors the freshly dissected whole brains of 10 to 25 frogs. Hundred mgm. samples were rapidly weighed from this homogeneous stock.

Blood samples were usually collected before breakfast. All blood samples were immediately defibrinated by shaking with glass perles for 5 minutes, and then promptly centrifuged. The supernatant serum was used for analysis. Only clear yellow sera were used and specimens with hemolysis were discarded. From each individual serum, specimens in triplicate were prepared as follows: 1 cc. of serum was mixed with 100 mgm. of frog brain, 1 cc. of Ringer's solution at pH 7.4, 1 cc. of Ringer's solution containing 3 mgm. of physostigmine salicylate, and 0.3 cc. of Ringer's solution containing 4.8 mgm. of glucose.

The Ringer's solution used in preparing mixtures for incubation was of the mammalian type and each 100 cc. contained 0.9 gram of NaCl, 0.4 gram of KCl, 0.011 gram of $CaCl_2$, and phosphate buffer (M/100) to give a pH value of 7.4. (The final concentration of the phosphorus was 0.03 gram per 100 cc. of Ringer's solution.) The pH of all mixtures was corrected to 7.4, the pH of blood, before incubation because the amount of acetylcholine synthesized depends on the pH.

Incubation. The mixtures were placed in vessels mounted on a shaking machine and shaken and incubated for 4 hours in a water bath. At short intervals through-

[1] This study was aided by a grant from the Josiah Macy, Jr. Foundation.

[2] Prostigmine (Hoffmann-La Roche).

[3] We are immeasurably indebted to Dr. Loewi for his enthusiastic interest in the development of the problem, and for his valuable advice on technic.

[4] The results were more uniform and small differences in the synthesis in the presence of serum were more readily demonstrable with frog brain than with mammalian brain.

TABLE I

Short summary of the clinical state of 8 patients with myasthenia gravis

Name	Sex	Age	Severity of disease	Duration	Thymectomy	X-ray treatment	Symptomatology	Neostigmine (Prostigmine Bromide Hoffmann-La Roche)		
								Dose before thymectomy	Dose	Achievement after medication
		years		*years*				*mgm. per day*	*mgm. per day*	
H	F	43	5+	15	Yes	Yes	Bedridden, severe lid ptosis, diplopia, constant difficulty in chewing and swallowing, very severe muscular fatiguability	225	120 to 180	Chews food, turns in bed, sits on chair
G	F	39	4+	3	No	Yes	Almost bedridden, severe lid ptosis, diplopia, difficulty in chewing and swallowing, severe muscular fatiguability		135	Sits in chair for a greater number of hours, can walk a few steps
R	F	23	3+	9	No	No	Moderate lid ptosis, occasional diplopia, occasional difficulty in chewing, moderate muscular fatiguability		90	Walks 1 to 2 blocks
Sa	F	32	3+	7	No	No	Moderate lid ptosis, occasional diplopia, occasional difficulty in chewing, moderate muscular fatiguability		90 to 150	Housework
S	M	22	2+	2	Yes	No	Slight lid ptosis, rare diplopia, moderate muscular fatiguability	120	15 to 45	Works as an inspector of machinery
V	F	20	2+	5½	No	No	Slight lid ptosis, rare diplopia, occasional difficulty in chewing, mild muscular fatiguability		45 to 75	Walks, goes to parties
P	M	36	2+	2	No	No	Difficulty in chewing, moderate muscular fatiguability		45 to 75	Walks, but unable to work
M	F	27	2+	6	No	No	Slight lid ptosis, rare diplopia, occasional difficulty in chewing, mild muscular fatiguability		15 to 45	Works as a cashier (against advice takes as high as 135 mgm. for almost normal function)
N*	F	47	1+	12	No	No	Mild muscular fatiguability			Housework

* Doubtful.

out the incubation, the gas above the mixtures was replaced by oxygen. Incubations were made at 23° C. and at 38° C.

After 4 hours of incubation, 1.2 cc. of distilled water were added to the samples to convert the osmotic pressure to that of frog Ringer's solution and the volume was made up to 10 cc. with frog Ringer's solution. (The Ringer's solution of the frog type contained 0.67 gram of NaCl, 0.02 gram of KCl, and 0.015 gram of $CaCl_2$ in 100 cc.) The pH was corrected to 6.8, and kept constant throughout all following steps of the analysis. (Both the magnitude of the contraction of the rectus abdominis muscle and the stability of the acetylcholine before and during boiling are modified by variations in the pH.)

The mixtures were then centrifuged. After centrifuging, the acetylcholine content of the supernatant fluid was ascertained by measuring the magnitude of contractions of the sensitized rectus abdominis muscle of the frog.

Preparation of the rectus abdominis muscle. The rectus abdominis muscle of the frog was excised, immersed in Ringer's solution, and kept in an ice-box (2° C.) for 2 hours to promote uniform responses of the muscle. The muscle was then suspended in a muscle chamber of 10 cc. capacity (method of Riesser (8) and Chang and Gaddum (9)), and sensitized by immersion for half an hour in Ringer's solution (at room temperature) containing 2 mgm. physostigmine salicylate per 100 cc. Thereupon, the physostigmine solution was replaced for 2 minutes by a solution containing 10 μgm. acetylcholine per 100 cc. The magnitude of contraction was registered by an isotonic lever on a kymograph. The muscle was then immersed for 5 minutes in Ringer's solution, followed by 5 minutes in a solution of physostigmine, and then again by immersion in the above acetylcholine solution. The procedure was repeated until 3 successive exposures to the solution of acetylcholine gave similar responses. (Between all subsequent immersions in any contraction inducing solution, the muscle was washed for 5 minutes with Ringer's solution and for 5 minutes with the Ringer's solution containing physostigmine.) The writing lever of the kymograph was so

weighted as to bring the contraction within the submaximal range where the muscle is sensitive to small changes in the concentration of acetylcholine.

These preliminary steps completed, the muscle was immersed for 2 minutes in one of the unknown solutions. Then, after measuring the contraction induced by the unknown solution, the muscle was immersed in sequence in a series of standard acetylcholine solutions, of concentrations covering the range of the unknown solutions. The acetylcholine content was thus ascertained for each unknown solution.

Assay of acetylcholine synthesis. The acetylcholine content of the mixtures, enumerated in Table II, was assayed.

It has been suggested (10, 11) that boiling brain tissue makes available the intracellular bound acetylcholine. Therefore these assay procedures were repeated after boiling. The residue of brain previously removed by centrifugation was suspended in the diluted supernatant fluid used in the above described procedure, and the whole boiled for 2 minutes at pH 6.8. It was then re-centrifuged and the supernantant fluid assayed for acetylcholine content by measuring the magnitude of contraction of the rectus abdominis muscle of frog as described above.

The stability of the muscle was tested before and after the assay of each unknown solution by immersion in standard acetylcholine solutions.

The acetylcholine content of each mixture was assayed on 2 to 12 different rectus abdominis muscle preparations. Thus, calculations on acetylcholine synthesis in each individual serum specimen were based on results obtained from at least 5 muscles.

Demonstration that the contractions induced by the mixtures are due to acetylcholine. To be certain that the contraction was induced by acetylcholine contained in the unknown solution and not by other substances, the muscles were immersed in the same unknown solution before and after being sensitized by immersion in physostigmine. Since only acetylcholine effects are significantly modified by physostigmine, it was inferred that any contraction that was augmented by physostigmine was due to acetylcholine.

Test for the occurrence of a substance that interferes with the action of acetylcholine on the rectus abdominis muscle. There is a possibility that during the 4 hours of incubation, other substances were produced in unequal quantities in the mixtures containing frog brain-control serum and frog brain-serum from patients with myasthenia gravis. Some of these substances might prevent acetylcholine from inducing contraction of the rectus abdominis muscle. If so, it is conceivable that the magnitude of the contraction of the rectus abdominis muscle on immersion in the incubated mixtures is the result of 2 effects: (*a*) the contraction inducing action of acetylcholine, and (*b*) the effect of an agent which prevents the acetylcholine from acting on the muscle. To investigate this possibility, we incubated for 4 hours mixtures containing frog brain, glucose, and control serum, or serum from a patient (H) with myasthenia gravis, but not containing physostigmine. In these mixtures, the acetylcholine synthesized was destroyed by the choline esterase present. Therefore, the incubated mixtures did not induce contraction of the rectus abdominis muscle. After incubation, the mixtures were diluted to 10 cc., as described above, and rectus abdominis muscles were immersed in these solutions for 30 minutes and afterwards immersed for 2 minutes in solutions of acetylcholine of known concentrations. It was found that neither mixture ((*a*) frog brain-control serum and (*b*) frog brain-serum from patient H with myasthenia gravis) modified the expected effect of acetylcholine. Therefore, it may be assumed that in such incubated mixtures, no substance was developed in sufficient concentration to prevent acetylcholine from acting on the rectus abdominis muscle of the frog.

Calculation of synthesis of acetylcholine. The acetylcholine synthesis within the incubated mixtures was calculated by subtracting from the acetylcholine content of the incubated mixtures the acetylcholine content of identical non-incubated mixtures. Comparisons were made between the acetylcholine synthesis resulting from the mixtures containing control serum, and those containing the serum from patients with myasthenia gravis.[5]

[5] The method employed for the assay of acetylcholine, as with all bioassays of this type, gave considerable variation in the results obtained in individual experiments. However, a sufficient number of experiments were performed in each group to assure the significance of the difference reported between the effect of control serum and serum from patients with myasthenia gravis.

TABLE II

Assay of acetylcholine synthesis

State	Frog brain	Ringer's solution	Serum	Physostigmine salicylate		Glucose	
					Final concentration		Final concentration
	mgm.	*cc.*	*cc.*	*mgm.*	*grams per 100 cc.*	*mgm.*	*grams per 100 cc.*
IA. non-incubated	100	3.3	0	3	0.09	4.8	0.15
IB. non-incubated	100	2.3	1	3	0.09	4.8	0.15
IIA. incubated	100	3.3	0	3	0.09	4.8	0.15
IIB. incubated	100	2.3	1 (control serum)	3	0.09	4.8	0.15
IIC. incubated	100	2.3	1 (myasthenia gravis)	3	0.09	4.8	0.15

RESULTS

Series 1. A comparison of the effect of control serum and serum from patients with myasthenia gravis on acetylcholine synthesis

I. Fresh non-incubated mixtures

A. Frog brain—Ringer's solution. The acetylcholine content of mixtures of frog brain-Ringer's solution, as assayed before boiling, averaged 0.39 μgm. and, as assayed after boiling, averaged 0.80 μgm. per 100 mgm. frog brain (Table III).

B. Frog brain-serum. Mixtures containing frog brain-control serum and frog brain-serum from patients with myasthenia gravis gave less constant results but, when averaged, the acetylcholine content was approximately the same as that of frog brain-Ringer's solution mixtures (Table III).

II. Incubated mixtures

A. Frog brain-Ringer's solution. The amount of acetylcholine synthesized at 23° C. by a mixture of frog brain-Ringer's solution, as assayed before boiling, averaged 0.70 μgm. and, as assayed after boiling, averaged 1.50 μgm. acetylcholine per 100 mgm. of frog brain. The amount of acetylcholine synthesized at 38° C., as assayed before boiling, averaged 0.41 μgm. acetylcholine, and, as assayed after boiling, averaged 1.02 μgm. acetylcholine per 100 mgm. of frog brain (Tables IV and V).

B. Frog brain-control serum. Five healthy persons and 59 patients with diseases other than myasthenia gravis served as controls. The patients had the following diseases: multiple sclerosis, brain tumor, cerebral accident, paresis, encephalitis, peripheral neuritis, ruptured disk, arterial hypertension, subacute bacterial endocarditis, secondary anemia, uremia, asthma, diabetes, lead poisoning, "gastro-enteritis," hyperthyroidism, myalgia, and undiagnosed muscular weakness. One hundred and two separate blood samples were collected and incubated in triplicate. The amount of acetylcholine synthesized at 23° C. by a mixture of frog brain-control serum, as assayed before boiling, averaged 1.16 μgm. acetylcholine, and as assayed after boiling, averaged 1.73 μgm. acetylcholine per 100 mgm. of tissue. The amount of acetylcholine synthesized at 38° C., as assayed before boiling, averaged 1.45 μgm. acetylcholine, and as assayed after boiling, averaged 2.08 μgm. acetylcholine per 100 mgm. of tissue. The amount of acetylcholine synthesized in the presence of serum of healthy subjects and of the patients with diseases

TABLE III

Acetylcholine content of freshly prepared non-incubated mixtures expressed in μgm. per 100 mgm. of tissue

Mixtures containing frog brain and	Assays of non-boiled mixtures				Assays of boiled mixtures			
	No. of separate mixtures	No. of rectus abdominis muscles	Acetylcholine content		No. of separate mixtures	No. of rectus abdominis muscles	Acetylcholine content	
			Mean	S. E.			Mean	S. E.
Ringer's solution	40	157	0.39	±0.011	20	59	0.80	±0.022
Serum	40	157	0.38	±0.029	20	59	0.82	±0.035

TABLE IV

Acetylcholine synthesis during 4 hours' incubation in mixtures containing frog brain and Ringer's solution expressed in μgm. per 100 mgm. of frog brain

Assays of non-boiled mixtures (38° C.)				Assays of boiled mixtures (38° C.)				Assays of non-boiled mixtures (23° C.)				Assays of boiled mixtures (23° C.)			
No. of incubated mixtures	No. of rectus abdominis muscles	Acetylcholine synthesis		No. of incubated mixtures	No. of rectus abdominis muscles	Acetylcholine synthesis		No. of incubated mixtures	No. of rectus abdominis muscles	Acetylcholine synthesis		No. of incubated mixtures	No. of rectus abdominis muscles	Acetylcholine synthesis	
		Mean	S.E.			Mean	S.E.			Mean	S.E.			Mean	S.E.
		μgm.				μgm.				μgm.				μgm.	
22	60	0.41	±0.013	28	55	1.02	±0.033	9	14	0.70	±0.023	9	14	1.50	±0.044

TABLE V

Comparison of the amounts of acetylcholine synthesized in the mixtures containing: 1. Frog brain—Ringer's solution; 2. Frog brain—control serum; and 3. Frog brain—serum from patients with myasthenia gravis

Average of the acetylcholine synthesized during 4 hours of incubation expressed in µgm. per 100 mgm. of frog brain

Mixtures containing frog brain, glucose, physostigmine, and	Assays of mixtures			
	Non-boiled 23° C.	Boiled 23° C.	Non-boiled 38° C.	Boiled 38° C.
Ringer's solution	0.70	1.50	0.41	1.02
Control serum	1.16	1.73	1.45	2.08
Myasthenia gravis serum				
H +++++*	0.47	0.75	0.53	0.90
G ++++			0.65	
R +++	0.59	0.92	0.79	1.11
Sa +++			0.82	1.16
S ++			0.88	
M ++			0.91	
P ++			0.91	1.29
V ++	0.75	1.10	0.92	1.30
N +?	0.93	1.38	1.17	1.69

* Most seriously ill patient: +++++; least seriously ill patient: +.

other than myasthenia gravis deviated from the average not more than ±15 per cent and usually less than ±10 per cent.

C. Frog brain-serum from patients with myasthenia gravis. The synthesis of acetylcholine in the mixtures of frog brain and serum from seriously incapacitated patients with myasthenia gravis was less than half that found in mixtures of frog brain-control serum. The amount of acetylcholine synthesized at 23° C. by mixtures of frog brain and serum from patient H with severe myasthenia gravis, as assayed before boiling, averaged 0.47 µgm. acetylcholine, and, as assayed after boiling, averaged 0.75 µgm. acetylcholine per 100 mgm. of tissue. The amount of acetylcholine synthesized at 38° C., as assayed before boiling, averaged 0.53 µgm. acetylcholine, and, as assayed after boiling, averaged 0.90 µgm. acetylcholine per 100 mgm. of tissue (Tables V and VI).

The most serious decrease in the synthesis of acetylcholine was noted in the mixtures containing serum from the patients most seriously ill with myasthenia gravis. The samples from the patient with the least impairment (N), constituting a borderline myasthenic state, presented a defect in synthesis barely below the level of some of the controls (Tables V and VI).[6]

[6] While this paper was in press, results similar to those of case S were obtained using serum of 3 additional and comparable patients with myasthenia gravis.

TABLE VI

Acetylcholine synthesized during 4 hours' incubation in mixtures containing frog brain and serum from patients with myasthenia gravis expressed in µgm. per 100 mgm. of tissue

Subject	Assays of non-boiled mixtures (38° C.)								Assays of boiled mixtures (38° C.)							
	No. of separate blood samples	No. of incubated mixtures	No. of rectus abdominis muscles	Acetylcholine synthesis: Minimum-maximum	Mean	S.E.	Deviation in percentage of average	Percentage of control †	No. of separate blood samples	No. of incubated mixtures	No. of rectus abdominis muscles	Acetylcholine synthesis: Minimum-maximum	Mean	S.E.	Deviation, percentage of average	Percentage of control
				µgm.	µgm.							µgm.	µgm.			
H +++++‡	11	32	85	0.42 to 0.61	0.53	±0.024	−20/+15	36.6*	1	2	6	0.86 to 0.95	0.90	±0.017	−5/+5	43.2**
G ++++	4	12	27	0.60 to 0.75	0.65	±0.010	−8/+15	44.9								
R +++	4	12	46	0.70 to 0.90	0.79	±0.012	−11/+14	54.6	4	12	24	1.02 to 1.19	1.11	±0.014	−8/+7	53.4
Sa +++	3	9	20	0.76 to 0.88	0.82	±0.013	−7/+7	56.6	3	9	20	1.08 to 1.23	1.16	±0.016	−7/+6	55.7
S ++	4	12	40	0.82 to 0.94	0.88	±0.010	−7/+7	60.8								
M ++	1	3	6	0.87 to 0.95	0.91	±0.014	−5/+4	62.9								
P ++	1	3	6	0.86 to 0.94	0.91	±0.018	−6/+3	62.9	1	3	6	1.20 to 1.36	1.29	±0.028	−7/+5	62.1
V ++	1	2	6	0.88 to 0.95	0.92	±0.016	−4/+3	63.6	1	2	6	1.22 to 1.34	1.30	±0.026	−6/+3	62.5
N +?	4	12	21	1.07 to 1.23	1.17	±0.015	−9/+5	80.8	4	12	24	1.49 to 1.80	1.69	±0.031	−12/+6	81.3
	(23° C.)								(23° C.)							
H +++++	1	2	6	0.42 to 0.52	0.47	±0.022	−11/+11	40.5***	1	2	6	0.70 to 0.81	0.75	±0.022	−7/+8	43.3****
R +++	4	12	30	0.54 to 0.64	0.59	±0.010	−8/+8	50.9	4	12	30	0.86 to 1.02	0.92	±0.014	−7/+11	53.0
V ++	1	2	6	0.69 to 0.80	0.75	±0.024	−8/+7	64.6	1	2	6	1.05 to 1.16	1.10	±0.024	−4/+5	63.5
N +?	2	8	20	0.87 to 0.97	0.93	±0.011	−6/+4	80.1	2	8	20	1.30 to 1.43	1.38	±0.015	−6/+4	79.8

† The percentage of control is calculated by taking the average amount of acetylcholine synthesized in the serum of controls as 100 per cent: * 1.45; ** 2.08; *** 1.16; **** 1.73.

‡ Most seriously ill patient: +++++; least seriously ill patient: +.

Incubated mixtures containing serum collected before and after the administration of 15 mgm. neostigmine bromide by mouth to 2 healthy adults, yielded similar acetylcholine content. All patients with myasthenia gravis were taking neostigmine bromide during these experiments. Blood samples were collected both before and after the morning medication. No significant difference between the 2 sets of blood samples was noted as regards acetylcholine synthesis.

Series 2. The effect on acetylcholine synthesis of the mixture of the control serum and the serum from patients with myasthenia gravis

A mixture of the serum of controls and the serum from patients with myasthenia gravis synthesized at 38° C. an amount of acetylcholine approximately equivalent to the sum of the synthesis of the 2 sera.

Series 3. Effect of the dialysate of serum on the synthesis of acetylcholine

To ascertain whether the factors which modify the synthesis of acetylcholine in the frog brain *in vitro* are dialyzable, serum was dialyzed through a semi-permeable cellophane membrane. Three cc. of serum were placed in a flat glass container (4 cc. volume), separated from another similar glass container by a cellophane membrane. This second vessel contained 3 cc. of Ringer's solution (mammalian type) and the apparatus was shaken for 4 hours at 23° C. The effect of the dialysate on the acetylcholine synthesis was then compared with the effect of Ringer's solution: 2 cc. of dialysate and 2 cc. of Ringer's solution were added to mixtures of frog brain. glucose, and physostigmine, incubated, and assayed as described above. The results are summarized in Table VII.

Frog brain mixed with 2 cc. of dialysate obtained from control sera, synthesized 0.68 μgm. acetylcholine at 38° C. The amount of acetylcholine synthesized at 38° C. in mixtures containing frog brain-Ringer's solution averaged 0.41 μgm. acetylcholine per 100 mgm. of frog brain. An increase of acetylcholine synthesis can best be demonstrated at 38° C., a temperature at which the difference between synthesis in mixtures of frog brain-Ringer's solution and frog brain-control sera is greater than at 23° C. The results indicate that at least some of the potentiator substances in the serum are dialyzable.

TABLE VII

Acetylcholine synthesized during 4 hours' incubation in mixtures containing 1. Frog brain and Ringer's solution; 2. Frog brain and dialysate of control serum; and 3. Frog brain and dialysate of serum from patients with myasthenia gravis expressed in μgm. per 100 mgm. of frog brain (assays of non-boiled mixtures)

Temperature	Mixtures containing frog brain and	No. of expts.	Amount of acetylcholine synthesis	
			Mean	S.E.
38° C.	Ringer's solution	22	0.41	±0.013
38° C.	Dialysate from controls	5	0.68	±0.049
23° C.	Ringer's solution	9	0.70	±0.023
23° C.	Dialysate from R (myasthenia gravis)	5	0.55	±0.038

To ascertain whether sera from patients with myasthenia gravis contain dialyzable substances that decrease the acetylcholine synthesis, mixtures of frog brain-dialysate of sera from patients with myasthenia gravis and mixtures of frog brain-Ringer's solution were incubated at 23° C., a temperature favorable for acetylcholine synthesis in mixtures containing frog brain and Ringer's solution and therefore suitable for demonstrating a depression. The amount of acetylcholine synthesized in mixtures containing frog brain-Ringer's solution averaged 0.70 μgm. acetylcholine. Mixtures containing frog brain and 2 cc. dialysate obtained from the serum of patient R with myasthenia gravis synthesized, on the average, 0.55 μgm. acetylcholine. The results suggest that at least some of the substances responsible for the decrease of the acetylcholine synthesis in patients with myasthenia gravis are dialyzable. Although it appears that there is contained in the serum of patients with myasthenia gravis an agent that inhibits acetylcholine synthesis, it may not be inferred that such agent is absent in the control sera.

DISCUSSION

These data reveal that the serum from patients with myasthenia gravis is an unfavorable medium for the synthesis of acetylcholine. The acetylcholine synthesis in mixtures of frog brain-Ringer's solution-control serum is almost 3 times as great as in mixtures of frog brain-Ringer's

solution-serum from a patient seriously incapacitated by myasthenia gravis (12).

This difference is significant, since variation from subject to subject, both among the healthy and among those with diseases other than myasthenia gravis, was less than ±15 per cent, and it was usually under ±10 per cent. The difference in acetylcholine synthesis between samples of serum of different patients with myasthenia gravis was considerable, but the variation in the sample of serum of any one myasthenic patient was less than ±15 per cent. The serum from patient H with severe myasthenia gravis was examined over a long period during which the clinical state changed slightly. This may have a bearing on the wider variation (−20, +15 per cent from the average) observed in this patient. Hence, the usually more than 100 per cent greater synthesis of acetylcholine with control serum, as compared with the serum from patients seriously incapacitated by myasthenia gravis, represents a major difference in the nature of the serum samples.[7]

Moreover, this reduction of acetylcholine synthesis is not an expression of debility, cachexia, immobility, or prostration.

It is of interest that the magnitude of the decrease in acetylcholine synthesis is related to the severity of the myasthenia gravis in this small group of patients. The serum from patients with the more serious clinical disability supported acetylcholine synthesis less well than did serum of patients in a better clinical state. With the method now employed, the determinable reduction in the less seriously ill is so small that obviously this technic is not of value for diagnostic purposes.

[7] The spinal fluid from patients with myasthenia gravis exerts a similar effect on the synthesis of acetylcholine to that of the serum (13).

While this paper was in press, Stoerk and Morpeth (14), using rat brain as a source of enzyme, reported that they could detect no difference in the amounts of acetylcholine synthesized in the presence of serum from control subjects and serum from patients with myasthenia gravis. Since they were also unable to demonstrate any difference in the amounts of acetylcholine synthesized in the presence of control serum as compared to Locke's solution, it is inferred that their adaptation of the method of Quastel, Tennenbaum, and Wheatley was not adequate for the demonstration of such differences in the synthesis of acetylcholine as are relevant to this discussion.

Since the dialysate of serum affects acetylcholine synthesis, it may be inferred that some of the modifying agents, both potentiators and depressors, are of a relatively small molecular size and not colloidal in nature.

Since acetylcholine synthesis is greater in frog brain-Ringer's solution-control serum mixtures than in frog brain-Ringer's solution mixtures, it is likely that the serum furnishes additional substrate to the enzyme responsible for acetylcholine synthesis. Also, a temperature of 38° C., that interferes with acetylcholine synthesis in frog brain-Ringer's solution mixtures, permits, in frog brain-Ringer's solution-serum mixtures, more synthesis than is found at 23° C. Apparently at 38° C., this particular enzyme is more active than at 23° C., and though the substrate contributed by frog brain is converted at 38° C. into a state less suitable for acetylcholine synthesis, the substrate added by the serum compensates for the loss.

The decrease in acetylcholine synthesis demonstrated in these *in vitro* studies suggests that there may be similar defects in acetylcholine synthesis in patients with myasthenia gravis. There is reason to assume that the serum of patients with myasthenia gravis which poorly supports acetylcholine synthesis *in vitro* acts similarly *in vivo*.

It seems probable that in patients with myasthenia gravis, the acetylcholine available at the synapses eventually becomes insufficient for effective and repeated contraction of the muscle. Neostigmine aids these patients by impeding the breakdown of the diminished quantities of acetylcholine.

SUMMARY

1. Using frog brain-serum and frog brain-Ringer's solution mixtures as preparations for studying rate of acetylcholine synthesis, and the sensitized rectus abdominis muscle of frog for bioassay of acetylcholine content of the mixtures, the effect of control sera and sera from patients with myasthenia gravis on acetylcholine synthesis was investigated.

2. In frog brain-Ringer's solution mixtures, acetylcholine synthesis is greater at 23° C. than at 38° C., while the reverse is true of frog brain-serum mixtures.

3. Acetylcholine synthesis in frog brain-control serum mixtures or in frog brain-dialysate of control serum mixtures exceeded that from frog brain-Ringer's solution mixtures.

4. Acetylcholine synthesis in mixtures of frog brain and serum from patients with myasthenia gravis was significantly less than that in frog brain-control serum mixtures. The more severe the myasthenia gravis, the less the acetylcholine synthesis. In mixtures of frog brain and dialysate of serum from patients with myasthenia gravis, acetylcholine synthesis was less than in mixtures of frog brain-Ringer's solution.

5. These results suggest that there is a defect in acetylcholine synthesis in patients with myasthenia gravis which can explain the fatiguability and weakness of these patients.

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CHANGES IN PLASMA VOLUME AND CARDIAC OUTPUT FOLLOWING THE INTRAVENOUS INJECTION OF GELATIN, SERUM, AND PHYSIOLOGICAL SALINE SOLUTION [1]

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There has recently been a renewed interest in the use of gelatin as a substitute for blood plasma (1 to 5). The purpose of the present study is to determine, in normal dogs, the degree to which a single large intravenous injection of gelatin solution, physiological saline solution, or serum remains in the vascular bed and to determine the effect of these injections on cardiac output.

METHODS

Dogs were anesthetized with an intravenous injection of sodium barbital, 250 mgm. per kgm. body weight. A tracheotomy was performed and a tracheal cannula inserted. A cephalic vein, carotid artery, and a small branch of the right and left femoral arteries were exposed and cannulated. Arterial blood pressure was recorded by means of a Hg manometer that was connected to the carotid cannula. The cardiac output, right auricular pressure, and hematocrit were determined and, at the same time, 10 to 40 mgm. of the dye, T-1824, were injected intravenously to determine the plasma volume. One hour later, 50 cc. per kgm. body weight of warmed gelatin solution, physiological saline solution, or serum were injected into the cephalic vein at the rate of 5 cc. per kgm. per minute. Twenty minutes after the completion of the fluid injection, the cardiac output was determined, an "indirect" determination of plasma volume was made, and the hematocrit was determined. At 40 minutes after the completion of the injection, an "indirect" determination of plasma volume was made and the hematocrit was determined. At 60 minutes after the completion of the injection, the right auricular pressure, hematocrit, and cardiac output were determined, and an "indirect" determination of plasma volume was made. At the same time, 10 to 20 mgm. of dye were injected for a second "direct" determination of plasma volume. Two hundred and forty minutes after the completion of the injection, the hematocrit, right auricular pressure, and cardiac output were determined. At the same time, 10 mgm. of dye were injected intravenously for a third "direct" plasma volume determination.

The plasma volume was determined by the method of Gibson and Evans (6). Arterial blood samples were drawn from a small branch of the femoral artery for determination of plasma dye content with a Klett-Summerson photoelectric colorimeter. The first sample was taken 20 minutes after the dye injection and 4 subsequent samples were taken at 10-minute intervals.

Cardiac output was determined by means of the Fick formula. The rate of oxygen consumption was measured with a modified Benedict Roth type apparatus. This was connected to the dog by means of a tracheal cannula. Blood was collected over Hg without contact with air by the method of Austin *et al.* (7). Arterial blood was drawn from a small cannulated branch of a femoral artery. Mixed venous blood was drawn, in most experiments, from the right auricle by means of a glass cannula that passed into the right auricle by way of the right external jugular vein, and in others, from the right ventricle according to the method of Marshall (8). The oxygen content of the blood was determined on 2 cc. samples by the method of Van Slyke and Neill (9).

In most experiments, right auricular pressure was measured immediately before each cardiac output determination by means of a water manometer that was connected to the cannula which passed into the right auricle. At the end of each experiment, the position of the tip of the cannula in the auricle was determined and all right auricular pressures were referred to this level as zero.

The hematocrit was determined on arterial blood by the method of Wintrobe (10).

Clotting was prevented in blood samples by drawing the blood into tubes which contained 1.3 mgm. dry ammonium oxalate and 0.7 mgm. dry potassim oxalate per cc. of blood. All blood withdrawn from the animal was replaced by transfusion of an equal volume of blood from another dog.

The gelatin[2] (Lot B78–1) used in these experiments was supplied as calcium gelatinate, produced by hydrolysis of alkali-treated bovine long bone collagen. A 3.75 per cent gelatin solution was prepared by dissolving the gelatin in 0.9 per cent sodium chloride solution, and adjusting the pH to 7.4 by adding sodium hydroxide. The gelatin

[1] Aided in part by a grant from the Knox Gelatine Company. The gelatin was furnished by the Knox Gelatine Company.

[2] A preliminary report of this work was presented at a conference on gelatin which was convened by the subcommittee on blood substitutes of the National Research Council, in Washington, D. C., Feb. 23, 1943.

solution was autoclaved for 90 minutes at 10 pounds pressure before using. The colloidal osmotic pressure of the gelatin solution was 29 mm. of Hg, as measured by the method of Hepp (11).

Pooled serum was prepared from 2 to 4 dogs and refrigerated for approximately 24 hours before being used.

The blood volume (B.V.), oxygen content of 100 cc. of arterial red blood cells (O_2 R.B.C.), and total peripheral resistance (T.P.R.), were calculated as follows:

$$\text{B.V.} = \frac{\text{P.V.}}{\text{P.H.}} \times 100$$

$$O_2\ \text{R.B.C.} = \frac{\text{A.O.}}{\text{R.H.}} \times 100$$

$$\text{T.P.R.} = \frac{\text{Pr.}}{\text{C.O.}} \times 1332$$

P.V. is plasma volume. P.H. is the percentage of plasma in the hematocrit. R.H. is the percentage of erythrocytes in the hematocrit. A.O. is the oxygen content of 100 cc. of arterial blood. Pr. is mean arterial pressure in mm. Hg. C.O. is cardiac output in cc. per second.

In some experiments the following supplementary data were obtained:

(*1*) The plasma protein content was determined by the gravimetric method (12).

(*2*) The plasma gelatin content was determined as hydroxyproline by the method of Macfarlane and Guest (13).

OBSERVATIONS

The results of all observations are tabulated in Tables I, II, and III.

TABLE I

The effect of intravenous physiological saline solution, serum, and gelatin solution on various circulatory factors

Expt. no.	Solution injected	Weight of dog	Time	Plasma volume	Hematocrit	Blood volume	Oxygen		Oxygen consumption	Cardiac output	Arterial pressure	Right auricular pressure	Arterial oxygen	Total peripheral resistance
							Arterial	Venous						
	cc.	*kgm.*	*minutes*	*cc.*	*per cent red cells*	*cc.*	*cc. per 100 cc. blood*		*cc. per minute*	*L. per minute*	*mm. Hg*	*mm. water*	*cc. per 100 cc. red cells*	*A.U.*
1	0.9 per cent NaCl 750	15.0	0	916*	22.1	1188								
			20	979	16.6	1204								
			40	933	18.4	1167								
			60	781										
			60	869*										
			240	915*	21.95	1146								
2	0.9 per cent NaCl 775	15.4	0	887*	47.3	1718								
			20	1058	40.0	1782								
			40	1080										
			60	965										
			60	939*	42.4	1659								
			240	843*	46.0	1591								
3	0.9 per cent NaCl 925	18.5	0	794*	58.1	1965	26.72	18.58	144	1.77	146	−2; +18	46	6593
			20	1077	50.0	2200	23.36	19.83	155	4.38	124	+18	47	2253
			40	986	51.1	2063								
			60	981										
			60	900*	51.6	1902	24.15	17.75	150	2.34	130	+27	47	4475
			240	820*	57.0	1953								
4	0.9 per cent NaCl 1075	21.5	0	964*	47.7	1905	22.24	17.53	138	2.93	158	0	47	4307
			20	1269	40.7	2140	19.45	14.65	176	3.67	162	+26	48	3532
			40	1143	45.4	2135								
			60	1029										
			60	1088*	44.8	2012	22.06	15.21	181	2.63	139	−14	49	4219
			240	847*	49.3	1722								
5	0.9 per cent NaCl 1350	27.0	0	1389*	51.5	2914	23.52	17.60	121	2.04	86	0	46	3372
			20	1952	39.0	3254	18.41	16.44	112	5.67	127	+1	47	1790
			40	1747	41.4	3005								
			60	1623										
			60	1770*	41.4	3072	19.62	16.02	108	2.97	98	+3	48	2637
			240	1517*	46.2	2845								

Effect of intravenous injection of gelatin solution, physiological saline, and serum on plasma volume, hematocrit, blood volume, arterial oxygen content, venous oxygen content, oxygen consumption, cardiac output, arterial pressure, right auricular pressure, total peripheral resistance, and arterial oxygen content of red blood cells. In all experiments, zero time represents the control determination. The other determinations are at 20, 40, 60, and 240 minutes after the completion of the fluid injection. L., liters. A.U., absolute units having the dimensions, dynes seconds $cm.^{-5}$.

* Plasma volume determination by the "direct" method. All other plasma volume determinations were by the "indirect" method.

TABLE I—*Continued*

Expt. no.	Solution injected	Weight of dog	Time	Plasma volume	Hema-tocrit	Blood volume	Oxygen		Oxygen con-sumption	Cardiac output	Arterial pres-sure	Right auricular pressure	Arterial oxygen	Total periph-eral resist-ance
							Arterial	Venous						
	cc.	*kgm.*	*min-utes*	*cc.*	*per cent red cells*	*cc.*	*cc. per 100 cc. blood*		*cc. per minute*	*L. per minute*	*mm. Hg*	*mm. water*	*cc. per 100 cc. red cells*	*A.U.*
6	Serum 740	14.8	0	798*	49.2	1578	23.47	14.55	90	1.01	110	−10; +25	48	8655
			20	1485	35.3	2315	15.49	9.07	90	1.41	52	−25; −15	44	2957
			40	1430	38.0	2321								
			60	1272										
			60	1092*	39.1	1807	17.47	13.31	87	2.10	89	−45; −40	45	3388
			240	1088*	40.8	1857	18.46	13.60	81	1.67	102	−25; +2	45	4891
7	Serum 1040	20.8	0	816*	58.1	2023	26.32	20.20	144	2.36	150	−7	45	5079
			20	1572	41.2	2720	19.42	15.61	150	3.94	131	−2	47	2653
			40	1489	45.2	2742								
			60	1558										
			60	1274*	46.3	2411	20.70	17.38	140	4.20	142	−5	45	2677
			240		52.3		23.02	19.02	155	3.88	166	+8	44	3420
8	Serum 785	15.7	0	679*	49.2	1372	21.74	18.45	64	1.93	144	−6; +4	44	5946
			20	1236	36.1	1971	16.63	13.55	110	3.57	114	−4; +1	46	2549
			40	1198	37.9	1961								
			60	1158										
			60	806*	38.6	1334	17.02	13.86	97	3.08	124	−4; +1	44	3212
			240	953*	38.6	1570	18.29	10.33	77	0.96	70	−11; +3	47	5810
9	Serum 685	13.7	0	589*	53.1	1267	24.61	20.42	77	2.11	148	−10; +25	46	5610
			20	1062	50.8	2194	17.20	12.97	96	2.27	82	0; +20	34	2893
			40	1032	52.4	2143								
			60	1028										
			60	746*	55.8	1700	21.43	15.82	91	1.62	115	−26; −21		5682
			240	603*	56.1	1396	23.82	17.10	77	1.14	129	−24; −18	42	9046
10	Serum 805	16.1	0	654*	40.2	1104	17.99	14.30	115	3.12	176	+6; +24	45	4515
			20	1277	28.6	1802	10.82	6.45	96	2.86	96	−56	38	2685
			40	1227	34.7	1890								
			60	1129										
			60	934*	40.1	1560	31.96	8.97	103	2.06	108	−37; −22	35	4188
			240	676*	45.5	1257	17.55	10.57	109	1.57	130	−7; 0	39	6633
11	Gelatin 570	11.4	0	651*	39.6	1083	18.81	12.59	64	1.03	101	−17; −10	48	7864
			20	1119	21.6	1431	10.72	7.93	68	2.44	124	−18; +12	50	4060
			40	1086	22.9	1412								
			60	947										
			60	1020*	26.5	1388	12.78	8.81	67	1.69	114	−10; −4	48	5394
			240	807*	34.1	1233	15.63	10.67	113	2.28	126	−4; +10	46	4419
12	Gelatin 968	19.4	0	846*	48.8	1687	21.11	16.88	142	3.35	144	−4; +1	43	3429
			20	1621	26.8	2219	12.93	10.54	151	6.31	136	+24; +28	48	1726
			40	1513	27.9	2095								
			60	1482										
			60	1639*	26.4	2246	13.05	9.92	124	3.96	134	+28	49	2701
			240	1267*	34.0	1948	15.52	12.04	150	4.23	132	0	46	2493
13	Gelatin 915	18.3	0	953*	48.4	1922								
			20	1759	32.8	2646								
			40	1672	36.4	2640								
			60	1558										
			60	1143*	39.5	1931								
			240	878*	48.7	1770								
14	Gelatin 670	13.4	0	599*	52.8	1289	23.68	21.27	89	3.69	128	−3; 0	45	2773
			20	1282	35.2	1988	16.82	14.92	142	7.50	145	−12; +15	48	1542
			40	1216	36.0	1904								
			60	1205										
			60	1052*	36.9	1676	18.04	14.98	101	3.32	142	−17	49	3420
			240	1075*	46.9	2024	22.17	17.51	100	2.14	135	−20; −8	47	5059

TABLE I—*Continued*

Expt. no.	Solution injected	Weight of dog	Time	Plasma volume	Hema-tocrit	Blood volume	Oxygen		Oxygen con-sumption	Cardiac output	Arterial pres-sure	Right auricular pressure	Arterial oxygen	Total periph-eral resist-ance
							Arterial	Venous						
	cc.	*kgm.*	*min-utes*	*cc.*	*per cent red cells*	*cc.*	*cc. per 100 cc. blood*		*cc. per minute*	*L. per minute*	*mm. Hg*	*mm. water*	*cc. per 100 cc. red cells*	*A.U.*
			0	819*	48.1	1586	24.05	20.83	74	2.29	124		50	4313
			20	1681	28.8	2361	13.84	11.50	99	4.24	133		48	2509
15**	Gelatin	18.0	40	1619	27.8	2243								
	900		60	1551										
			60	1361*	29.0	1929	13.92	11.16	96	3.47	126		48	2893
			240	1000*	36.8	1598	16.72	12.28	99	2.23	134		45	4803
			0	922*	48.5	1812	20.80	16.14	111	2.37	132		43	4443
			20	1857	23.2	2433	10.73	7.89	120	4.22	133		46	2509
16**	Gelatin	20.7	40	1716	25.7	2315								
	1010		60	1636										
			60	1504*	26.6	2052								
			240	1351*	30.0	1959	13.35	8.87	139	3.10	117		45	3021
			0	864*	42.2	1507	16.39	13.25	67	2.13	128		39	4803
			20	1760	16.2	2105	7.66	5.73	76	3.93	141		47	2869
17**	Gelatin	21.5	40	1683	17.5	2064								
	1000		60	1595										
			60	1333*	19.0	1654								
			240	961*	27.4	1341	12.91	8.90	71	1.76	122		47	5554
			0	909*	48.6	1778	21.63	18.64	89	2.97	190	−12; 0	45	5107
			20	925	50.8	1903	22.64	16.03	92	1.39	162	−22; −4	45	9334
18	Control	22.8	40	921	50.6	1884								
			60	931										
			60	909*	51.8	1893	22.47	15.78	90	1.34	168	−25; +2	43	10005
			240	1000*	53.9	2176	23.17	15.73	111	1.49	152	−20; +3	43	8168

** Mixed venous blood drawn from the right ventricle. In all other experiments, mixed venous blood was drawn from the right auricle. Blood that was withdrawn was not replaced by transfusion from another dog.

The observed values for plasma volume indicated a discrepancy in the values at 60 minutes as determined by the "direct" and "indirect" methods. The average percentage by which the plasma volumes by "indirect" determination exceeded those by "direct" determination was 28 with serum, 11 with gelatin. With saline solution, the 2 values differed by only 2 per cent, which is not a real difference. For determination of the plasma volume by the "direct" method, the only requirement is that a dye concentration-time curve be obtained which, when extrapolated back to the time of injection, will give the dye concentration which would have been present if instantaneous mixing had occurred. Shifts of dye or fluid do not interfere with the satisfaction of this requirement, provided that the rates at which these occur remain constant during the period of determination. The "indirect" method of determination of plasma volume requires that the actual rate of removal of dye from the plasma be determined during the control period, and that this rate continues until the time that this "indirect" determination is made.

The probable errors in the "indirect" determination which might explain the discrepancy appeared to be (*1*) reduction in the optical density of the plasma, apart from its content of T-1824, and (*2*) increased rate of removal of dye. The use of large amounts of dye (6) makes the first inadequate as an explanation. This was established by determination of the light transmission of serum, gelatin, and dye solutions. The second explanation is supported by the demonstration of Gregerson and Rawson (14, 15) of the binding of T-1824 by plasma proteins. If the theoretical dye concentration at 60 minutes is calculated on the basis that the accelerated rate of departure of plasma protein as observed produces an equal acceleration of departure of T-1824, it agrees with the observed concentration within 6 per cent. However, the escape of fluid *per se*, as 0.9 per cent NaCl, apparently does not

TABLE II

Effect of intravenous gelatin solution and serum on plasma proteins and plasma gelatin concentration

Substance	Expt. no.	Time after injection	Plasma protein	Plasma gelatin	Circulating plasma protein	Circulating plasma gelatin	Injected serum: Protein	Injected serum: Colloidal osmotic pressure
		minutes	*grams per 100 cc.*	*grams per 100 cc.*	*per cent of control*	*per cent of amount injected*	*grams per 100 cc.*	*mm. Hg*
Serum	6						5.09	20.3
	7						5.45	24.3
	8						5.81	
	9	0	6.71		100		5.33	19.3
		240	6.80		103			
	10	0	6.90		100		5.09	20.6
		60	6.38		132			
		240	6.80		102			
Gelatin	12	60		0.70		36		
		240		0.80		31		
	13	60		0.71		27		
		240		0.83		24		
	14	0	6.04	0.00	100	0		
		60		0.83		39		
		240	2.92	0.90	87	43		
	16	0	6.28		100			
		20	3.14					
		60	3.33		87			
		240	3.84		90			
	17	0	6.50		100			
		20	2.76					
		60	3.23		77			
		240	3.34		57			

At zero time, the control determinations are given. Other determinations were made at 20, 60, and 240 minutes after the completion of the fluid injection. The experiments in this table having the numbers corresponding to numbers in Table I were on the same animal.

accelerate the departure of the dye, since the "direct" and "indirect" determinations agree. In the case of gelatin, the increase in loss of dye is apparently associated with the amount of plasma protein, rather than gelatin, that leaves the blood.

The figure for plasma volume at 60 minutes obtained by the "direct" method seems more nearly to represent the true plasma volume, so that it was used in calculation of the blood volume, the percentage of change in plasma volume, and the percentage of retention of fluid. Since only "indirect" determinations of plasma volume were made at 20 and 40 minutes, these are given in the tables, although they are probably too high in the experiments with serum and gelatin.

Saline. Five experiments were performed. The plasma volume and blood volume were elevated at 20 and 40 minutes after injecting the 0.9 per cent sodium chloride solution but had returned nearly to the control value after 240 minutes. The plasma volume at 20, 40, 60, and 240 minutes after the completion of the fluid injection was always much less than the expected plasma volume if all of the injected solution had remained in the circulation. In each experiment, the cardiac output was increased above the control at 20 minutes after the completion of the fluid injection, the average value being plus 117 per cent. After one hour, it had returned toward the control value. The total peripheral resistance was markedly decreased at 20 minutes following the fluid injection and returned toward the normal at 60 minutes after the injection. Following the injection, there was no marked effect on arterial pressure. Right auricular pressure was slightly elevated in 2 experiments and there was no change in the third. The oxygen content of 100 cc. of red cells was increased in each experiment, the average value being 2.4 per cent above the control, at 20 minutes after the completion of the injection.

Serum. Five experiments were performed with serum. The plasma volume was increased after the serum injection and had returned to a point above the control after 240 minutes. The amount of the injected serum that remained in the vascular bed was much greater at 60 and 240 minutes than the amount of saline solution that remained (Table III). In 4 of these experiments, the cardiac output was increased between 7 and 84 per cent above the control at 20 minutes after the injection. After 4 hours, the cardiac output had returned toward the control value. In 1 dog, the cardiac output decreased throughout the experiment. The reason for this is not clear,

TABLE III

Average percentage of change in various circulatory factors after giving saline, serum, and gelatin

Time	Solution injected	0	20 minutes (percentage of change)	40 minutes (percentage of change)	60 minutes (percentage of change)	240 minutes (percentage of change)
Plasma volume	Saline	990 cc.	+ 27	+19	+11	− 1
	Serum	707 cc.	+ 89	+80	+36	+21
	Gelatin	808 cc.	+ 96	+86	+61	+32
Percentage of injected solution that remained in circulation	Saline	975 cc.	+ 26	+18	+10	− 1
	Serum	811 cc.	+ 76	+70	+32	+26
	Gelatin	862 cc.	+ 90	+81	+57	+30
Blood volume	Saline	1938 cc.	+ 8	+ 5	+ 1	− 5
	Serum	1469 cc.	+ 52	+53	+21	+14
	Gelatin	1555 cc.	+ 40	+36	+19	+11
Cardiac output	Saline	2.25 L. per minute	+117		+22	
	Serum	2.11 L. per minute	+ 38		+37	− 4
	Gelatin	2.48 L. per minute	+ 96		+36	+19
Total peripheral resistance	Saline	4757 A.U.	− 44		−19	
	Serum	5961 A.U.	− 52		−32	+ 6
	Gelatin	4604 A.U.	− 45		−16	+ 1
O_2 content of 100 cc. of arterial R.B.C.	Saline	41.6 cc.	+ 2.4		+ 3.8	
	Serum	45.7 cc.	− 8.5		− 7.5	− 4.6
	Gelatin	44.8 cc.	+ 8.1		+ 5.1	+ 4.1

The average of the control values for the various factors are given in the zero column. The average percentage of increase (+) above the control and the average percentage of decrease (−) below the control of the various factors at 20, 40, 60, and 240 minutes after the completion of the fluid injection are given. The total peripheral resistance is expressed in absolute units (A.U.) having the dimensions $\frac{\text{dynes sec.}}{\text{cm.}^5}$. R.B.C., red blood cells.

but the fact that, at autopsy, this animal was found to have no pericardium may have significance. The arterial pressure fell markedly after the completion of the injection of the serum in 3 experiments and fell slightly in the other 2. It then returned toward the normal level. The total peripheral resistance was markedly reduced following the fluid injection and then returned toward the normal level. The right auricular pressure was less than the control value after the injection of serum in 2 experiments, and slightly greater than the control in 2 experiments. The oxygen content of 100 cc. of red blood cells was decreased in most of the experiments, the average value being a decrease of 8.5 per cent below the control, at 20 minutes after the completion of the injection.

Gelatin. Seven experiments were performed with gelatin. The plasma volume was increased after the injection and returned to a point above the control value after 240 minutes. The amount of the injected gelatin solution that remained in the vascular bed was much greater at 60 and 240 minutes than the amount of saline solution that remained (Table III). The cardiac output was increased between 78 and 138 per cent above the control value at 20 minutes after the completion of the injection and had returned toward the control value after 4 hours. There was no marked effect on arterial pressure, although there was a small rise in 6 of the experiments at 20 minutes after the injection. The total peripheral resistance was markedly reduced following the fluid injection and then returned toward the normal value. In the 3 experiments in which auricular pressure was measured, it was elevated at 20 minutes after the completion of the injection. The oxygen content of 100 cc. of red blood cells was increased in 5 of the 6 experiments in which it was measured, the average increase above the control being 8.1 per cent, at 20 minutes after the injection.

DISCUSSION

The results reported here are in agreement with those of others who have shown that physiological saline solution quickly leaves the vascular bed after intravenous injection (16, 17), that

serum is retained in the vascular bed for several hours (16, 18, 19), and that gelatin solution is retained in the vascular bed for several hours (4, 20, 21). The results show that the gelatin solution is just as effective as serum in maintaining plasma volume in the normal vascular bed. Although there was, on the average, a greater percentage of retention in the vascular bed of the gelatin solution than there was of the serum, this does not necessarily mean that gelatin solution is more effective as a blood plasma substitute than serum because the colloidal osmotic pressure of the 2 solutions was different. The gelatin solution had a colloidal osmotic pressure of 29 mm. of Hg while the serum had a colloidal osmotic pressure that ranged between 19.3 and 24.3 mm. of Hg. Thus, if the gelatin molecules stay in the vascular bed as well as the serum protein molecules do, it would be expected that a larger volume of the gelatin solution would stay in the vascular bed under these experimental conditions. From the data presented here, we do not know how effective the gelatin solution would be in maintaining the plasma volume in an abnormal vascular bed as in shock, but the use of gelatin in the treatment of shock (5) and in experimental hemorrhage (1 to 3) suggests that gelatin is effective in maintaining plasma volume in abnormal vascular beds.

Since blood volume and cardiac output were determined simultaneously, the results were examined for the presence of any consistent relationship between these 2 values. In 4 of the 14 experiments, a linear relationship was found, with cardiac output increasing as the blood volume increased. In 10 experiments, no consistent quantitative relationship was found, although in most cases the cardiac output was increased when the blood volume was increased. The possible errors interfering with the demonstration of a relationship include (*1*) erroneous values for blood volume, particularly at 20 minutes after the injection of the fluid, due to use of the "indirect" method for plasma volume, and the hematocrit for calculation of blood volume, and (*2*) erroneous values for cardiac output arising from the method, or changes in the cardiac output from influences other than an increase in blood volume. In 7 out of 10 control experiments, of which experiment 18 (Table I) is an example, the first output was higher than subsequent ones. The average of these determinations showed that the second output was less than the first by 33 per cent. The cause of this is not clear but may be due to the fact that the animals were anesthetized with sodium barbital. Blalock (22, 23) has pointed out that the cardiac output in dogs anesthetized with sodium barbital is variable and that the cardiac output during the first 90 minutes of anesthesia is generally higher than subsequent determinations. The reason for this is not clear but it may be due to the depth of the anesthesia. Since large volumes of fluid were given intravenously in each experiment, there was a tendency for the concentration of the anesthetic in the animal to be reduced as a result of the fluid injection. In most of our experiments, an attempt was made to overcome this objection by adding sodium barbital to the injected solution in a quantity that was calculated to maintain the concentration of sodium barbital constant in the animal. There is also some question as to whether mixed venous blood is obtained when blood is withdrawn from the right auricle. In some unpublished observations that we have made, it appears that in some animals, blood from different points in the right auricle may vary in oxygen content.

In spite of the fact that in control experiments the cardiac output had a tendency to be variable, the cardiac output was increased in all experiments except one at 20 minutes after the injection of fluid. This increase was greatest with saline solution, and least with serum. The mechanism responsible for this increase in cardiac output and the cause of the different degrees of increase in cardiac output when different solutions were injected are not clear. However, the increase in cardiac output was associated with a decrease in total peripheral resistance, and the decrease in peripheral resistance probably contributed to the increase in cardiac output by increasing the venous return to the heart. The decrease in peripheral resistance may have been the result of dilatation of peripheral blood vessels, the opening up of peripheral blood vessels that had been closed, a decrease in the viscosity of the blood, or any combination of these factors. Since no measurement of blood viscosity was made, we are unable to determine how much of

the decrease in total peripheral resistance was due to viscosity change and how much was due to dilatation of blood vessels. However, the injection of saline solution would be expected to cause the viscosity of the blood plasma to decrease most, the gelatin next, and the serum least, and this is the relative order of effectiveness of these 3 solutions in increasing cardiac output. A decrease in the red blood cell concentration of the blood causes a decrease in blood viscosity. This factor would tend to cause the greatest decrease in viscosity in the gelatin experiments and the least decrease in the saline experiments.

In all of these experiments, fluid was leaving the vascular bed for 4 hours following the fluid injection. It is generally thought that the factors regulating this removal of fluid from the circulation are (*1*) the difference between the hydrostatic pressure on the inside of the capillaries and that in the tissue spaces, and (2) the difference between the colloidal osmotic pressure on the inside of the capillaries and that in the tissue spaces. The 0.9 per cent sodium chloride solution had no colloidal osmotic pressure; therefore, when injected, it would tend to decrease the colloidal osmotic pressure of the blood and this would tend to cause fluid to pass from the vascular bed through the capillary wall into the tissue spaces. Although the colloidal osmotic pressures of the serum and gelatin were not identical with that of plasma, these differences alone could not account for the fluid shifts observed. The alternative is that in the gelatin and serum experiments, the hydrostatic pressure in the capillaries was elevated and caused fluid to pass from the blood through the capillary wall into the tissue spaces.

The data for plasma volume and plasma protein indicate that, following the injection of blood serum, fluid and protein leave the circulation at such a rate that the protein concentration of the blood plasma remains constant. It is also shown that, following the injection of gelatin, the reduction in plasma protein content is not only from dilution, but also from disappearance of plasma protein from the circulation (average of 22 per cent lost at the 240 minute observation). The data for plasma gelatin indicate that much of it has left the circulation at 60 minutes (66 per cent of the amount injected), but suggest that the fraction remaining leaves slowly. It is of interest to note that in experiment 14, the colloidal osmotic pressure of the plasma at the 240-minute observation was maintained at 22.5 mm. Hg, a pressure actually higher than the value of 20.4 found before injection of the gelatin. A much lower pressure would be expected from the content of plasma protein (2.92 per cent) and gelatin (0.9 per cent) observed.

It was surprising to find that the auricular pressure changed so little when the changes in blood volume and cardiac output were so large after injecting the various solutions. In a few cases, the auricular pressure was decreased after the fluid injection, at which time the blood volume and cardiac output were increased. This may have been the result of a decrease in intrathoracic pressure or an unusually large dilatation of the vascular bed, thereby pooling blood and lowering the right auricular pressure.

It has been suggested (3) that "pseudo-agglutination" of erythrocytes after the injection of gelatin might interfere with their oxygenation. We obtained no figures on arterial percentage saturation, but have calculated the oxygen content of unit volume of erythrocytes in arterial blood as possibly bearing on this point. This value was not reduced by gelatin.

SUMMARY

Plasma volume, arterial and venous oxygen content, oxygen consumption, arterial blood pressure, right auricular pressure, and the hematocrit were determined in normal barbitalized dogs, before and after the intravenous injection of 50 cc. per kgm. body weight of 0.9 per cent sodium chloride solution, gelatin solution, and serum. Blood volume, cardiac output, total peripheral resistance, and the oxygen content of 100 cc. of arterial red cells were calculated.

Sodium chloride solution gave a small and brief increase in plasma volume. Gelatin solution and serum gave a greater and more sustained increase in plasma volume.

The injection of these solutions caused the cardiac output to increase and the total peripheral resistance to decrease.

No consistent quantitative relationship was found between blood volume and cardiac output, between blood volume and right auricular pres-

sure, or between cardiac output and right auricular pressure.

Blood serum and a 3.75 per cent gelatin solution are about equally retained in the vascular bed, both to a greater extent than is 0.9 per cent sodium chloride.

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THE INDIRECT MEASUREMENT OF MEAN VENOUS OXYGEN TENSION DURING ANOXIA

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Homeostatic mechanisms to compensate for the anoxia of high altitude are presented by several systems of the body and may be either rapid or delayed. Increased respiration and increased cardiac output, which tend to minimize the decrease of the pressure at which oxygen is delivered to the tissues, are immediate responses to oxygen want. In contrast, increased oxygen capacity of the blood is produced only after several days of acclimatization to anoxia, as in life on mountains. A third compensatory adjustment which has been suggested, but not demonstrated, involves changes in the intracellular enzymes such as to produce more efficient utilization of oxygen at lowered pressure. Of these three mechanisms, response to the acute oxygen want of aviation must rely essentially on the rapid respiratory and circulatory adjustments to diminish tissue anoxia. The single physiological datum which most sensitively reflects the extent of these adjustments is the mean venous oxygen tension ($MVpO_2$), which represents the average tension at which oxygen leaves the blood at the venous end of the capillaries.

The $MVpO_2$ has been measured directly by several observers (1, 2) on blood obtained by puncture of the right side of the heart or catheterization of the right auricle, but these methods are hardly suitable for widespread use. Indirect measurement, by equilibration of suitable gas mixtures with pulmonary arterial blood in the alveoli, has been reported by a number of observers (3 to 9) since first used by Plesch in 1909 (3). These investigations, which were concerned with measurement of cardiac output employing the Fick principle, have been reviewed in detail in Grollman's monograph (10). The methods used have been largely discarded during the past decade.

The possibility of application to the problems of aviation medicine and the availability of a simpler method of measuring oxygen tension have led us to reconsider the technic of indirect measurement of the $MVpO_2$. In seeking optimal conditions of equilibration and assuring the reliability of the method finally adopted, several thousand determinations of oxygen tension were made by a nearly instantaneous physical method with an accuracy of 0.5 mm. Hg. The data presented here are only a small part of those available to show the reproducibility of the results.

PART I—THE METHOD OF MEASUREMENT OF $MVpO_2$

Procedure

The following procedure was finally adopted. A Douglas bag with a wide-bore 3-way metal stopcock is filled with the desired gas mixture. The subject, wearing a nose-clip, draws 10 quiet breaths through a rubber mouthpiece connected by the stopcock with the open air. He then exhales completely to the air, draws a maximal inspiration from the bag, exhales completely to the air again, and draws another maximal inspiration. The mouthpiece is withdrawn and the subject exhales almost completely and closes his lips about the pinched stem of an empty rubber bladder, exhaling slightly at the time in order to prevent entrance of outside air. He then exhales completely into the bladder the remaining 200 to 400 cc. of air which can be expelled from the lungs. The above procedure is carried out as rapidly as possible, occupying no more than 5 seconds. The gas in the bladder is reinhaled and forcibly and completely exhaled at the normal respiratory rate of 3 seconds per cycle for a total of 4 rebreathings, and is immediately analyzed.

The proof of equilibration depends upon comparison of the pO_2 of the rebreathed mixture with the pO_2 of the mixture in the rinsed lungs before rebreathing. In all of the experiments on the author as subject, such data were obtained by alternating the above rebreathing procedure with an incomplete procedure ("pO_2 before rebreathing") extending only to the initial expulsion into the bladder. In most of the later experiments with untrained subjects, however, the subject exhaled into a small glass Y-tube connected to 2 empty bladders, 1 of which was immediately clamped off before rebreathing. This improved procedure not only halved the time necessary for a given series of determinations, but increased the value of the comparison

between rebreathed and unrebreathed mixtures, which were obtained simultaneously rather than alternately.

Consideration of conditions of equilibration

The lungs were rinsed with a gas mixture which would produce approximately the desired pO_2, but which contained little or no CO_2. Since the diffusion of CO_2 between blood and alveoli is very rapid, the above procedure was based on the expectation that a rebreathed N_2-O_2 mixture would reach approximate equilibrium with venous CO_2 and thereby permit O_2 equilibration at normal pH. This was substantiated in several experiments in which it was found that (*a*) the gas mixtures after 4 rebreathings contained CO_2 as expected at a partial pressure slightly above that of the normal alveolar air taken during the same experiment, and (*b*) the addition of 6 per cent CO_2 to the rinsing mixture produced no alteration in the value of pO_2 attained at equilibrium. These 2 observations justified the omission of CO_2 from the gas mixtures, so that the mixtures could be made up by simply partly filling Douglas bags with N_2 and then exhaling into the bag until the mixture was shown by rapid analysis to have attained the desired pO_2.

Most of the previously published methods involved rinsing the lungs with an appropriate mixture and then either holding the breath or rebreathing a rather large volume of gas. The results of our early experiments, however, showed that neither of these methods produced as close an approach to equilibrium as did rebreathing a small volume (200 to 400 cc.) of gas. It is obvious on theoretical grounds that equilibrium across the alveolar membrane will be more complete the smaller the sample, but a lower limit is imposed by the requirement that the sample reinspired from the bag be large enough to permit mixing of alveolar and dead space air. Evidence for the inadequacy of equilibration by holding the breath is presented below (Table I).

Uniform results were obtained only when a procedure was adopted for insuring a reasonably uniform physiological state. All the experiments were performed in the sitting position, within easy reach of all the apparatus, in order to minimize muscular activity between rebreathings. To allow for recovery from such slight exertion as was necessary, the routine was adopted of preceding each test by 10 quiet breaths through the mouthpiece. (The "basal" state was not practicable because the long intervals necessary to attain this state between tests would have severely restricted the number of analyses.) Although this procedure eliminated most of the scattering of the data, the values reflected the effect of the vigorous act of rebreathing and subsequent hyperpnea on the physiological state at the time of a repeat test a few minutes later. Thus successive tests with a low oxygen mixture caused a drop of 2 to 3 in $MVpO_2$, and of 10 mm. in alveolar pO_2. Reproducible results were attained if a steady state was produced by spacing tests 2 to 5 minutes apart.

Since no obvious change was observed following a meal, experiments were not limited to the post-absorptive state. A much more important variable, difficult to control, was nervous tension. A higher $MVpO_2$ appeared to be associated with a state of slight excitement or tension, as might be expected from the effect of sympathico-adrenal activity on cardiac output.

Evidence of equilibrium

In Table I is presented a representative series of 63 consecutive tests on the author as subject, with different rinsing mixtures at atmospheric pressure, spaced at approximately 3-minute intervals. It is apparent that rebreathing 2 times caused less complete adjustment toward the venous pO_2 than rebreathing 4 times, as would be expected; holding the breath was even less efficient than 2 rebreathings. Rebreathing 6 times caused a definite further drop when the mixture was too high, but little or none in the neighborhood of equilibrium. Thus of the 4 such tests with the 26, 20, and 14 mm. rinsing mixtures, 2 caused a rise and 2 a fall by a fraction of a millimeter compared with the preceding 4-breath (routine) test. This is considered adequate evidence of the absence of significant recirculation.

Table I indicates a $MVpO_2$ on that occasion of 29 mm. Hg. It appears from the data obtained with mixtures producing higher or lower initial pO_2 than 29 mm. that the process of rebreathing 4 times causes the mixture to arrive at a pO_2 approximately half-way from its initial

value to the true venous pO_2. Thus the 34 mm. mixture produced a pO_2 before rebreathing of 38.8 and a pO_2 after 4 rebreathings of 34.0 mm. Hg.

Application to previously untrained subjects

In order to test the applicability of the method to a variety of subjects, it was taught to 14 sailors, furnished through the cooperation of the U. S. Naval Medical Center, Bethesda, Maryland. These men varied widely in intelligence, physical development, and vital capacity; no cardio-respiratory abnormalities could be demonstrated by physical examination, x-ray of the chest, or electrocardiogram. None had had any previous experience as subjects in respiratory studies. As a rule, fairly consistent data were obtained after one day of practice, with further improvement during the subsequent 3 to 4 days of study. Two additional men were discarded after an unsuccessful trial for one day. The major source of error for most new subjects was admission of outside air to the rebreathed mixture, leading to grossly high values. After this had been eliminated, an appreciable fluctuation of the values persisted in some cases; only 3 or 4 of the subjects appeared capable of maintaining

TABLE I

Consecutive values at approximately 3-minute intervals of alveolar pO_2 (mm. Hg) after rinsing the lungs twice with mixtures of various O_2 tensions

pO_2 before rebreathing	pO_2 after 2 rebreathings	pO_2 after 4 rebreathings	pO_2 after 6 rebreathings
Rinsing mixture pO_2 = 40 mm.			
		35.0	
		35.5	
		35.8	
42.0			
	37.8		
		36.0	
			34.0
Holding breath 10 seconds without rebreathing = 38.2			
Rinsing mixture pO_2 = 34 mm.			
		34.0	
		34.0	
38.8			
	35.5		
		34.0	
			32.5
Holding breath 10 seconds without rebreathing = 36.5			
Rinsing mixture pO_2 = 30 mm.			
		33.2	
		32.2	
		32.5	
37.8			
34.0			
34.5			
	32.8		
		31.2	
Holding breath 10 seconds without rebreathing = 33.8			
Followed by 10 minutes respite to refill Douglas bag			
		35.8	
		32.0	
36.0			
34.2			
	32.0		
		30.8	
			30.5
		31.8	
35.8			

TABLE I—*Continued*

pO_2 before rebreathing	pO_2 after 2 rebreathings	pO_2 after 4 rebreathings	pO_2 after 6 rebreathings
Rinsing mixture pO_2 = 26 mm.			
		30.5	
		32.5	
		30.5	
32.2			
	31.8		
		29.0	
			29.2
Holding breath 10 seconds without rebreathing = 30.0			
Rinsing mixture pO_2 = 20 mm.			
		30.0	
		29.5	
30.0			
	29.2		
		28.8	
			28.0
31.0			
Holding breath 10 seconds without rebreathing = 28.0			
Followed by 3-hour intermission and lunch.			
Rinsing mixture pO_2 = 14 mm.			
		29.0	
		29.0	
26.0			
	27.8		
		27.8	
			27.0
After 15-minute rest = 28.5			
27.8			
26.2			
		28.0	
27.0			
			28.2
26.8			
Holding breath 10 seconds without rebreathing = 26.2			
		27.8	

as constant a steady state as was demonstrated in Table I by the author. The rinsing mixtures found necessary to produce equilibrium in the various subjects varied from 5 to 25 mm. pO_2, but in all cases, the adequacy of equilibration was assured by approaching it from both the high and the low side.

Table II presents a morning's results from one subject, using the improved procedure of obtaining a rebreathed and an unrebreathed sample from the same rinsing of the lungs. The $MVpO_2$ was quite constant at 27 mm. The values obtained with these subjects on several occasions lay within the narrow range of 26 to 30 mm. at atmospheric pressure and were 2 to 3 mm. lower at a simulated altitude of 12,000 feet.

TABLE II

Values of alveolar pO_2 (mm. Hg) of subject Bernier after rinsing the lungs twice with mixtures of various O_2 tensions at atmospheric pressure

Time	pO_2 before rebreathing	pO_2 after 4 rebreathings	Estimated $MVpO_2$
Rinsing mixture pO_2 = 12.5 mm.			
8:51	24.5	26.5	
8:54	22.6	26.0	
8:58	23.0	26.5	
9:03	21.4	24.5	>26
Rinsing mixture pO_2 = 22.0 mm.			
9:10	26.0	27.0	
9:15	26.2	27.5	
9:19	25.5	26.5	
9:23	25.5	26.5	
9:27	25.2	26.2	
9:32	25.2	26.0	>26.5
Rinsing mixture pO_2 = 24.5 mm.			
9:41	27.0	27.4	
9:46	26.2	26.8	
9:50	29.4	27.6	
9:56	27.2	27.0	
10:00	30.2	26.5	27.0
Rinsing mixture pO_2 = 31.0 mm.			
10:18	29.5	28.8	
10:24	30.5	28.8	
10:29	30.0	31.6	
10:35	29.4	28.4	
10:39	32.0	28.8	
10:43	39.5	32.0	<28

Discussion

The major theoretical objection to this method is the possibility of recirculation before equilibrium can be reached. The procedure of 2 rinsings followed by 4 rebreathings occupies a maximal total of 19 seconds. Although the circulation time, measured by tests using the tongue, respiratory center, or appearance of radioactive material as an end-point, is reported to be 12 to 18 seconds in normal subjects (11), this determination omits the distance from the end-point, adjacent to the capillaries, to the level of the venous system at which the test material was injected. This venous path is probably the slowest part of the route, since the cross-section of the system of venules is large in order to function under a very low pressure gradient. In addition, these tests measure the velocity of the most rapid portion of the blood stream, which had taken the shortest route; this may be well ahead of the statistically more significant *average* speed of recirculation. Evidence was presented above to show that significant recirculation was not present in our experiments, even if the rebreathing were prolonged to 6 breaths. Such a margin of safety is necessary if the method is to be valid under conditions of anoxia, which increases the speed of circulation.

It is felt that the gas mixtures produced by this technic approach true equilibrium with the venous (pulmonary arterial) blood. The values observed at atmospheric pressure in the author on various days ranged from 29 to 33 mm. Hg-pO_2. This is lower than most of the results reported by the direct methods, which ranged generally from 35 to 40 mm. This apparent inconsistency may be explained by several considerations: (*1*) the subject was not basal, the pulse rarely falling below 80; (*2*) the act of rebreathing at a level close to complete expiration involves more muscular effort than normal respiration; (*3*) the direct measurements, which are the ultimate standard, may be too high because of the accelerated circulation attendant upon the excitement of cardiac puncture; (*4*) most important, the intermittent exposures to anoxia and acid-base shifts caused by the rebreathing affected the respiratory and circulatory centers, as shown by the fall in alveolar pO_2 and $MVpO_2$. Since no studies of blood pH were made, it is impossible to say whether this change is due to

respiratory alkalosis induced during the period of hyperpnea upon recovery from the anoxia of the test, or to a direct effect upon the nervous centers. It is to be emphasized that this procedure furnishes reproducible but not basal results.

PART II—EXPERIMENTS AT SIMULATED HIGH ALTITUDE

Measurement of $MVpO_2$

Determinations on the author as subject were made with a variety of rinsing mixtures during exposure in a decompression chamber to a simulated altitude of 12,000 feet (U. S. Bureau of Standards—Standard Atmospheric Pressure Table). The results, which are not presented in detail, indicated an even better equilibration at this altitude than at atmospheric pressure. With a series of 6 rinsing mixtures, ranging from 18.5 to 39 mm. pO_2, the pO_2 before rebreathing varied from 22 to 32 mm., while the 16 values of pO_2 after rebreathing fell within the narrow limits of 24.2 to 27.5 mm. pO_2, with an $MVpO_2$ of 25.5. It was also noted that the "Ordinary Alveolar pO_2" (the last portion of a forcible expulsion following several normal breaths) fell from 53.5 on first reaching this altitude to a steady value of 46 to 48, 15 minutes later; during a series of rebreathing experiments, occasional alveolar samples ranged from 37 to 40 mm. It appears that both the early adjustment to altitude and the process of rebreathing affect the respiratory volume, as indicated by the alveolar pO_2.

On the basis of this satisfactory equilibration, tests were performed on the same subject at various simulated altitudes using only 1 or 2 gas mixtures at each altitude, since prolonged experiments are difficult under these conditions, and it did not seem indispensable to approach equilibrium from both directions at high altitude. At 16,000, 18,000, and 20,000 feet, the rebreathing caused a brief convulsion, but the subject was able without a single failure to carry out the procedure, taking pure O_2 immediately following the final expiration into the bladder. This refreshment was limited to 1 or 2 breaths of O_2, since it was desired to interfere as little as possible with the process of acclimatization to the diminished O_2 tension. The results are presented in Table III.

Calculation of cardiac compensation

The values for the Alv. pO_2 and $MVpO_2$ at the various altitudes, interpolated from the results in Table III, are assembled in Table IV. From these data, the corresponding percentage saturation of arterial and venous

TABLE III

Alveolar pO_2 with appropriate rebreathing mixtures at various simulated altitudes

Time	pO_2 before rebreathing	pO_2 after 4 rebreathings	Ordinary alveolar pO_2	Conclusion
Ground level—atmospheric pO_2 160, temp. 72° F., pulse 88				
10:00 a.m.			105	
			100	
			103	
Mixture pO_2 = 25 mm.				
		34.2		
	34.6			
		32.8		
	34.0			
		33.0		
	34.2			
			92	
			98	
			104	
		32.5		At altitude 0
			95	Alv. pO_2=90
			98	$MVpO_2$ =32.0
		32.0		
			88	
10:35 a.m.			89	
Altitude 8,000 feet—atmospheric pO_2 118, pulse 88				
Mixture pO_2 = 27.5 mm.				
			63.0	
			65.0	
		30.5		
	30.0			
		29.0		
	30.0			
		29.0		
			56.5	
			56.5	
Mixture pO_2 = 24.5 mm.				
		29.0		
	27.5			
		28.8		At 8,000 feet
			50.5	Alv. pO_2=55
			54.0	$MVpO_2$ =29.0
			58.5	
		29.2		
			55	

TABLE III—*Continued*

Time	pO_2 before re-breathing	pO_2 after 4 re-breathings	Ordinary alveolar pO_2	Conclusion
Altitude 12,000 feet—atmospheric pO_2 = 102, pulse 90				
Mixture pO_2 = 27.5 mm.				
11:30 a.m.			48.0	
			46.0	
			43.5	
		27.2		
	26.0			
		25.8		
			42.0	
			43.2	
		27.8		
	26.0			At 12,000 feet
			40.0	Alv. pO_2=40
		26.0		$MVpO_2$ =26.0
			39.2	
11:45 a.m.			39.5	
Altitude 16,000 feet—atmospheric pO_2 = 86, pulse 100				
Mixture pO_2 = 29.5 mm.				
			36.5	
			36.5	
	25.8			
		24.2		
			37.0	
Mixture pO_2 = 25.5 mm.				
		24.0		
	24.0			
		22.8		
			34.0	
		23.8		At 16,000 feet
			34.0	Alv. pO_2=34.0
			33.5	$MVpO_2$ =23.5
		23.2		
12:15 p.m.	23.5			

blood was derived from a standard oxygen dissociation curve, the mean of the 2 similar curves reported for AVB and GSA at pCO_2 40 mm. (12). The negligible correction for the lower affinity for oxygen of venous as compared with arterial blood was omitted. This difference may be interpolated from the nomogram of the blood of AVB (13) as amounting to less than 1.0 per cent saturation with a change from 35 to 45 mm. pCO_2, which exceeds the difference in pCO_2 between arterial and venous blood.

It is shown in Table IV that the A-V (arterio-venous) difference (recorded in terms of percentage saturation) falls progressively with increasing altitude. According to the Fick principle, cardiac output equals oxygen consumption divided by A-V difference in oxygen content. Since oxygen consumption is essentially unchanged by moderate anoxia (14), the cardiac output is inversely proportional to the A-V difference, and the ratio of the cardiac output at any altitude to that at ground level may be simply calculated as the inverse ratio of the respective A-V differences. The cardiac output increases steadily from 106 per cent of normal at 8,000 feet to 189 per cent at 20,000 feet.

The extent to which this increased circulation prevents tissue anoxia may be calculated as follows. If there were no circulatory change (0 per cent circulatory compensation), the A-V difference at any altitude would be the same as that at atmospheric pressure, and the venous percentage of saturation at any altitude would be the arterial percentage of saturation minus this fixed A-V difference (34 per cent saturation). Such calculated values are listed in Table IV (Uncompensated venous saturation), in which the following column lists the corresponding oxygen tensions (Uncompensated $MVpO_2$), calculated from the

TABLE III—*Continued*

Time	pO_2 before re-breathing	pO_2 after 4 re-breathings	Ordinary alveolar pO_2	Conclusion
Altitude 18,000 feet—atmospheric pO_2 = 80, pulse 94				
Mixture pO_2 = 24 mm.				
12:30 p.m.	22.0			
			32.5	
		22.0		
			30.0	
	21.8			
		22.0		
			31.0	
		21.8		At 18,000 feet
	22.0			Alv. pO_2=30.5
			30.2	$MVpO_2$ =21.8
			30.0	
1:00 p.m.		21.5		
Altitude 20,000 feet—atmospheric pO_2 = 72, pulse 98				
Mixture pO_2 not noted in record				
			30.0	
			31.0	
	21.5			
		21.5		
	21.0			
			28.0	
		19.0		
			28.0	
			28.0	
1:15 p.m.		19.8		At 20,000 feet
			28.0	Alv. pO_2=28.0
		20.0		$MVpO_2$ =19.8
Ground level, pulse 82				
Mixture pO_2 = 22.5 mm.				
1:30 p.m.	32.0			
			78.0	
			82.0	At altitude 0
		31.5		Alv. pO_2=78
			76.0	$MVpO_2$ =32.0
		32.0		

TABLE IV

Circulatory data at various altitudes

Altitude	Alveolar pO_2	MV pO_2	Arterial	Venous	A−V difference	Relative cardiac output	Uncompensated venous saturation	Uncompensated $MVpO_2$	Circulatory compensation
feet	*mm. Hg*		*percentage of saturation*			*per cent*	*per cent*	*mm. Hg*	*per cent*
0	80 to 90	32.0	97	63	34	100	63	32	
8,000	55	29.0	89	57	32	106	55	28	25
12,000	40	26.0	77	51	26	130	43	22.5	40
16,000	34.0	23.5	67	45	22	155	33	18	40
18,000	30.5	21.8	61	42	19	179	27	15.5	42
20,000	28.0	19.8	55	37	18	189	21	13.5	38

Alveolar pO_2 and $MVpO_2$ were determined at the various altitudes and the other data calculated from them. Uncompensated Venous Saturation and $MVpO_2$ represent the values which these functions would have had if the A-V difference had remained constant and the Alveolar pO_2 had varied as observed at each altitude.

standard dissociation curve. If, on the other hand, there were 100 per cent circulatory compensation, the venous percentage of saturation would remain constant at its normal value at atmospheric pressure (63 per cent) and the A-V difference would drop proportionately with decreasing arterial saturation. Actually, the venous percentage of saturation lies between the 2 extremes, and the extent of circulatory compensation may be calculated by the formula:

$$\text{Percentage of circulatory compensation} = 100 \times \frac{\text{Actual venous percentage of saturation} - \text{Uncompensated venous percentage of saturation}}{\text{Normal venous percentage of saturation} - \text{Uncompensated venous percentage of saturation}}$$

For example, at 12,000 feet the Venous percentage of saturation was 51, and the Uncompensated venous percentage of saturation would be $77 - 34 = 43$; the normal venous percentage of saturation was 63; the percentage of circulatory compensation was therefore $100 \times \frac{51 - 43}{63 - 43} = 40$ per cent. At all altitudes but the lowest, the calculated circulatory compensotion was about 40 per cent—*i.e.*, the lowering of venous percentage of saturation was 2/5 less than it would have been if the cardiac output were constant. The lower value (25 per cent) at 8,000 feet is not very significant, since the slight anoxia here caused so small a deviation of $MVpO_2$ from normal that the calculation of circulatory compensation was subject to a large error.

The circulatory compensation may also be evaluated by a similar formula applied to O_2 tensions rather than to saturations, with similar results. Translated into terms of an aviator's "ceiling," $MVpO_2$, and hence the tissue oxygen tension, at an altitude of 18,000 feet is that which would have been reached at 13,000 feet if there were no circulatory compensation.

The data of Table IV are also presented in Figure 1, in which the shaded areas indicate the extent to which circulatory compensation decreases anoxia.

Discussion

The calculation of cardiac compensation rests on the assumption that the gas in the alveoli is in equilibrium with the blood leaving the alveolar capillaries. On the basis of the evidence of equilibrium shown in Part I, this assumption seems justifiable for the equilibrium between $MVpO_2$ and venous blood. The question of the equilibrium between ordinary alveolar air and arterial blood, however, has long been debated, with general abandonment of the secretion theory. The more acceptable diffusion theory presupposes the existence of a gradient, however small, and therefore a higher value for alveolar than arterial pO_2. Dill, Christensen, and Edwards (15), on the Chilean expedition, found an average alveolar-arterial difference at an altitude of 4.7 km. of only about 2 mm., but Dill, Edwards, and Robinson (16) noted somewhat larger differences in subjects breathing nitrogen-oxygen mixtures or subjects in a chamber decompressed to an oxygen tension of 86 mm. Since we have not determined arterial saturation directly, we must assume that equilibrium exists.[1] It is likely that the error so introduced is small and

[1] Recent work by F. J. W. Roughton (personal communication) has indicated that the difference observed between measured arterial saturation and that predicted from alveolar pO_2 may be largely due to an artefact, caused by the presence in normal blood of appreciable quantities of methemoglobin. This appears to be rapidly reduced after the blood is drawn and thereby to increase the measured oxygen capacity and hence decrease the apparent saturation. It may be that the alveolar pO_2 is actually a better measure of arterial saturation than is the usual measurement of content divided by capacity.

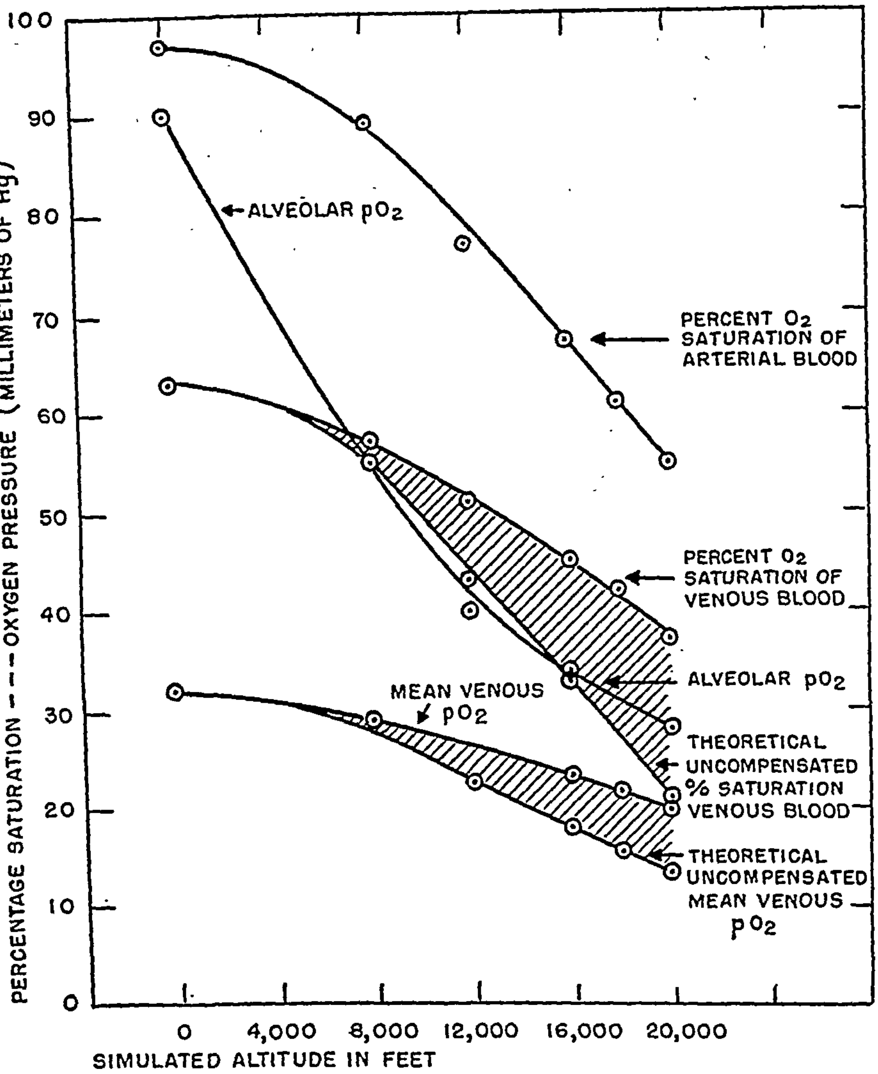

FIG. 1. CIRCULATORY DATA ON SUBJECT BDD AT VARIOUS SIMULATED ALTITUDES IN DECOMPRESSION CHAMBER

Alveolar pO_2 and $MVpO_2$ were determined and the other data calculated from them. The shaded areas represent the effectiveness of the increased cardiac output in diminishing tissue anoxia.

fairly constant in terms of percentage of saturation at various altitudes.

Another source of error in this calculation is the utilization of a standard oxygen dissociation curve. The largest deviations in the various reported curves occur in the lower portion, which corresponds to the values of $MVpO_2$ at high altitudes. If we choose 1 of the 2 curves of Bock, Field, and Adair (12), rather than the mean of the 2 curves as was done, the circulatory compensation values at the various altitudes are in the neighborhood of 33 per cent rather than 40 per cent. While the accuracy of the calculations is undoubtedly limited by these assumptions regarding alveolar-arterial equilibrium and the applicability of an O_2 dissociation curve obtained on another individual, it is felt that the order of magnitude of the calculated cardiac compensation, and its relative constancy at various altitudes, are valid derivations from the data. It may be noted that Grollman (14), measuring cardiac output by the acetylene method while

breathing air-N_2 mixtures, detected no decrease in A-V difference until the mixtures reached a pO_2 as low as 87 mm. (corresponding to *ca.* 16,000 feet).

The increases in cardiac output calculated above are well above the observed increases in pulse rate (10 to 20 per cent at 16 to 20,000 feet), indicating an effect of anoxia on stroke volume as well as on heart rate. Such an effect has been reported by Herbst and Manigold (17), who by an indirect sphygmographic method found an increase of stroke volume of 15 to 45 per cent and an increase of minute volume of 30 to 70 per cent in a number of subjects at simulated altitudes of 5 to 8,000 m. Matthes and Malikiosis (18) measured arterial saturation by a photoelectric colorimetric method across the ear lobe, and venous saturation by the same method after holding the lungs full of nitrogen until venous blood reached the ear capillaries. At a simulated altitude of 6,000 m., they found a doubling of minute volume, a 75 per cent increase in stroke volume, and a decrease of venous saturation to 54 to 61 per cent, from 68 to 72 per cent observed at atmospheric pressure. In contrast with these results, Keys *et al.* (19), using the less indirect technic of roentgenkymography, reported little or no increase in stroke volume with gas mixtures corresponding to an altitude of 18,000 to 28,000 feet.

It is to be emphasized that the indirect method of measuring $MVpO_2$ here presented involves considerable departure from the basal states but there appear to be no systematic errors to invalidate the comparison of values obtained at various altitudes or in different individuals. In 7 of 8 subjects, a correlation was noted between the $MVpO_2$ at 12,000 feet and resistance to loss of consciousness at higher altitudes. The values obtained with these and other normal individuals, however, fell within so narrow a range that it appears unlikely that very significant differences between subjects can be established.

SUMMARY

A method is presented for determining the mean venous oxygen tension ($MVpO_2$) indirectly by means of equilibrating gas mixture of low pO_2 with pulmonary arterial blood. Reproducible results were produced under standard but non-basal conditions, ranging in 15 individuals from 26 to 32 mm. Hg at atmospheric pressure.

Observations at simulated altitudes of 8,000 to 20,000 feet indicated that the cardiac output rose progressively with altitude, reaching 189 per cent of normal at 20,000 feet. This circulatory compensation decreased the tissue anoxia at the various altitudes by 40 per cent, the $MVpO_2$ at 20,000 feet being 19.8 mm. rather than the value of 13.5 mm. which would have obtained if the cardiac output were constant.

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A THERAPEUTIC AND PHARMACOLOGICAL STUDY OF SULFADIAZINE, MONOMETHYLSULFADIAZINE, AND DIMETHYLSULFADIAZINE IN LOBAR PNEUMONIA [1]

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The methyl salt of sulfathiazole was discarded because it produced peripheral neuritis (1). It was subsequently discovered that hens were sensitive indicators of this toxicity (2). However, only by trial in patients could the applicability of the avian observations to man be validated, though a shorter trial with less damage would have been possible had this avian test been known sooner.

Roblin and his coworkers (3) who prepared sulfadiazine also described its monomethyl and dimethyl homologues. When tested on hens, the toxicity for peripheral nerves found with the methyl salt of sulfathiazole was not encountered in the methyl compounds of sulfadiazine. Because of the absence of injury to the peripheral nerves, these new drugs have been offered for clinical investigation (4 to 6). New sulfonamides must be equally potent, mole per mole, to compete with or supplant their predecessors. Effective concentrations must be maintained by the same or less frequent administration. They must be less toxic and must be detoxicated to substances less likely to form crystal aggregates which may irritate or block the renal tubules. After animal trial, the effect on patients must be determined because the pharmacological properties may be different in the human or may be modified both by disease and by the temperature of the patients. Accordingly we have studied the effects of monomethylsulfadiazine [4] and dimethylsulfadiazine (7) in acute respiratory disease.

In vitro observations of the effect of equivalent concentrations of sulfadiazine, monomethylsulfadiazine, and dimethylsulfadiazine run in parallel

TABLE I

*Comparative blood concentrations with sulfadiazine, monomethylsulfadiazine, and dimethylsulfadiazine**

Drugs	Cases	Mgm. of "free" drug per 100 cc. of blood — Hours: 24	48	72
Sulfadiazine	1	5.8	8.1	6.5
	2	5.2	10.4	7.7
	3	6.7	4.9	8.0
	4	6.7	5.3	3.0
	5	7.0	10.9	10.1
	6	9.4	5.9	10.0
Monomethyl-sulfadiazine	1	6.0	11.0	**
	2	15.0	18.0	
	3	3.2	10.0	
	4	7.0	16.0	
	5	7.6	9.0	
	6	8.4	12.8	11.7
	7	7.8	6.8	6.6
	8	17.8	17.8	15.3
	9	8.8	11.2	
	10	17.2	18.8	13.8
	11	19.8	11.0	
	12	5.6	17.6	18.0
	13	10.0	7.0	
	14	9.2	16.0	10.2
	15	12.2	19.2	17.0
	16	14.4	19.8	16.4
	17	8.6	18.6	14.2
	18	15.8	12.0	14.2
Dimethyl-sulfadiazine	1	5.4	3.3	8.0
	2	8.4	10.8	6.3
	3	3.8	1.9	1.7
	4	5.5	3.8	1.6
	5	9.8	6.8	9.5
	6	8.9	9.6	4.2
	7	9.6	4.6	3.8
	8	5.5	9.2	1.0

* These drugs were given 4 grams *statim* and 1 gram every 4 hours day and night.

** Blood levels not taken where blank spaces appear.

[1] This study received additional support from the Metropolitan Life Insurance Co., and from Mr. Bernard M. Baruch, Mr. Bernard M. Baruch, Jr., Miss Belle N. Baruch, and Mrs. H. Robert Samstag.

[2] Deceased November 9, 1943.

[3] Brewer and Co., Inc., Research Laboratories, Worcester, Mass.

[4] We are indebted to Lederle Laboratories, Incorporated, Pearl River, New York, for generous supplies of these drugs.

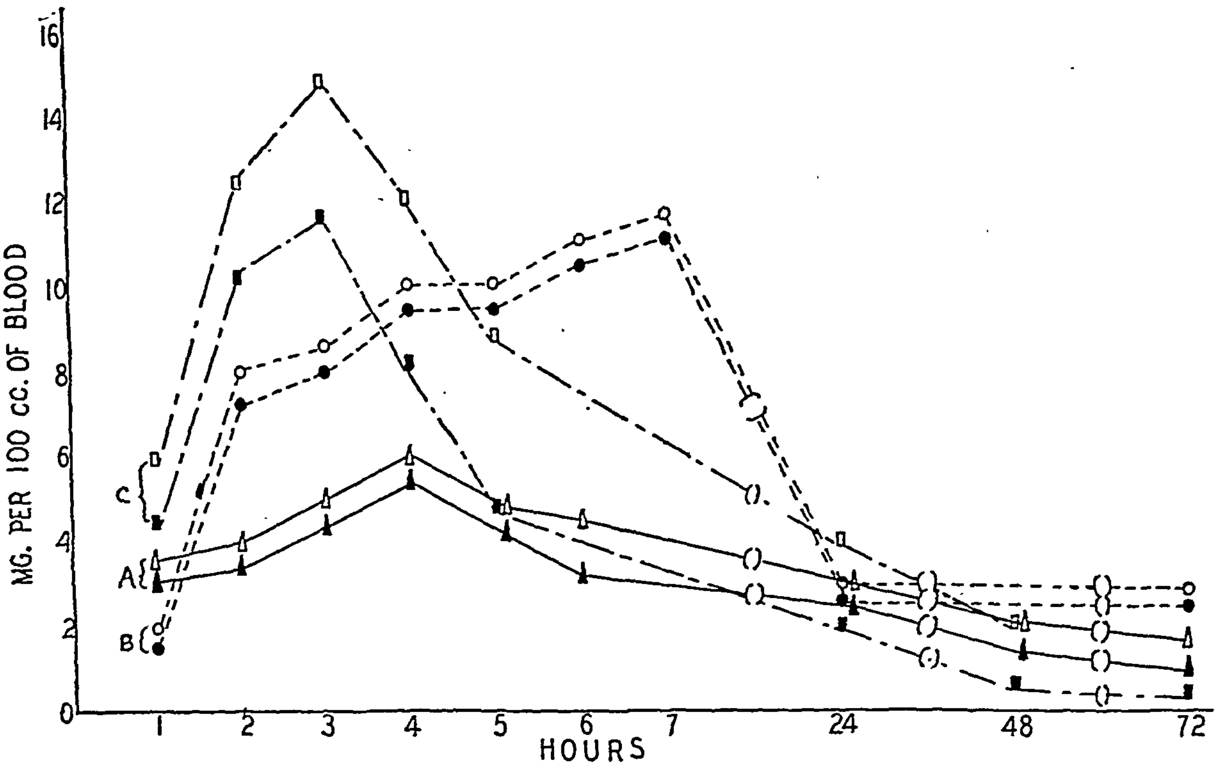

FIG. 1. AVERAGE CONCENTRATIONS OBTAINED IN THE BLOOD AFTER SINGLE ORAL 4 GRAM DOSES
A. Sulfadiazine (Average of 7 subjects)
B. Monomethylsulfadiazine (Average of 5 subjects)
C. Dimethylsulfadiazine (Average of 4 subjects)
Legend: □○△ = Total drug
■●▲ = Free drug

on a B. Friedlander B organism, a pneumococcus III, and a pneumococcus VI did not show significantly superior bactericidal action of the methyl salts.

PHARMACOLOGY

Sulfadiazine, monomethylsulfadiazine, and dimethylsulfadiazine are readily absorbed from the gastrointestinal tract. When they are administered orally, high concentrations may be obtained. High initial levels may be attained rapidly with all 3 drugs but they are maintained only with sulfadiazine and monomethylsulfadiazine (Table I).

When equal quantities are administered, monomethylsulfadiazine gives higher blood levels than sulfadiazine. The average concentrations of sulfadiazine, monomethylsulfadiazine, and dimethylsulfadiazine, obtained in the blood after single oral doses, are shown in Figure 1. In 7 subjects, at the end of 4 hours, a 4 gram dose of sulfadiazine resulted in blood levels ranging from 3.4 to 6.0 mgm. with an average of 5.2 mgm. of the free drug per 100 cc. In 5 subjects, at the end of 4 hours, a 4 gram dose of monomethylsulfadiazine resulted in blood levels ranging from 4.8 to 14.0 mgm. with an average of 9.4 mgm. of the free drug per 100 cc. Corresponding conjugated values were 0.5 to 2.2 mgm. with an average of 0.9 mgm. per 100 cc. of blood. Under similar conditions, free dimethylsulfadiazine in the blood reached concentration peaks varying from 6.0 to 11.7 mgm. with an average of 8.4 mgm. per 100 cc. The corresponding conjugated dimethylsulfadiazine values ranged from 1.0 to 4.3 mgm. with an average of 4.3 mgm. per 100 cc. of blood. With all the drugs, measurable amounts were found in the blood for approximately 72 hours.

When a *statim* dose of 4 grams was followed by 1 gram every 4 hours day and night, it was found that levels of sulfadiazine and monomethylsulfadiazine were well maintained. Blood concentrations for sulfadiazine ranged from 1.7 to 14.8

TABLE II

Comparative urinary recoveries of sulfadiazine and its mono and dimethyl analogues

Dose drug ingested	Conjugated drug in total recovery	Recovery		
		Free	Conjugated	Total
grams	*per cent*	*per cent*		
SULFADIAZINE				
2	7.7	60.0	5.0	65.0
2	31.6	65.0	30.0	95.0
3	73.3	13.3	36.6	49.9
3	41.6	23.3	16.6	39.9
3	22.0	23.3	6.6	29.9
3	40.9	43.3	30.0	73.3
5	40.9	26.0	18.0	44.0
5	28.0	36.0	14.0	50.0
5	66.6	20.0	40.0	60.0
5	33.3	40.0	20.0	60.0
5	33.3	28.0	14.0	42.0
11	28.8	33.6	13.6	47.2
14	44.7	41.4	33.5	74.5
22	69.6	11.3	25.9	37.2
MONOMETHYLSULFADIAZINE				
4	43.7	45.0	35.0	80.0
4	51.5	40.0	42.5	82.5
4	50.0	42.5	42.5	85.0
4	41.3	42.5	30.0	72.5
18	75.0	20.0	60.0	80.0
18	19.6	70.0	17.2	87.2
19	58.6	32.6	46.3	78.9
DIMETHYLSULFADIAZINE				
4	20.0	70.0	17.5	87.5
4	48.4	42.5	40.0	82.5
4	40.0	45.0	30.0	75.0
4	31.1	57.5	26.0	83.5
20	47.9	44.0	40.5	84.5
26	55.3	18.9	23.4	42.3
28	58.8	38.5	55.0	93.5
31	74.5	22.9	67.1	90.0
38	47.8	41.1	39.7	80.8
41	78.8	16.6	61.9	78.5
45	83.8	15.1	78.6	93.7

mgm. per 100 cc.; of this amount, 10 to 13 per cent was in the conjugated form. Blood levels for monomethylsulfadiazine ranged from 3.2 to 23.0 mgm. per 100 cc. and approximately 18 per cent was in the conjugated state.

The administration of dimethylsulfadiazine by the same regimen resulted in high initial levels which were not satisfactorily maintained. Following a peak, the concentrations diminished in many instances to as low as 1.0 or 2.0 mgm. per 100 cc. of blood. Continuing the dose of 1 gram every 4 hours did not again elevate the level of dimethylsulfadiazine. In some cases, the levels were fairly well maintained.

In each of the 3 drugs, following a single dose, approximately 50 per cent of the total amount recovered in the urine was excreted in the first 24 hours. In 72 hours, the amount of sulfadiazine (free and conjugated) recovered from the urine varied from 30 per cent to 95 per cent. For monomethylsulfadiazine (free and conjugated), the variation was 72 to 87 per cent. Dimethylsulfadiazine (free and conjugated) varied from 73 to 85 per cent.

About 35 per cent of the amount of sulfadiazine recovered from the urine was conjugated, of monomethylsulfadiazine 50 per cent, and of dimethylsulfadiazine 45 per cent. The total amount of sulfadiazine recovered from the urine ranged from 11.3 to 65 per cent of the free and 6.6 to 40.0 per cent of the conjugated drug. For monomethylsulfadiazine, the variations were 20.0 to 70.0 per cent of the free and 17.2 to 60.0 per cent of the conjugated. For dimethylsulfadiazine, it was 15.1 to 70.0 per cent of the free and 17.5 to 78.6 per cent of the conjugated (Table II).

TOXICITY

In the 14 sulfadiazine, 7 monomethylsulfadiazine, and 11 dimethylsulfadiazine cases studied pharmacologically, fluid intake was not forced. The patients however, took water freely. The daily urinary volume in all cases varied from 770 to 1800 cc. In a number of patients treated with dimethylsulfadiazine, there was a marked polyuria up to 4000 cc. Nausea and vomiting occurred in about 10 per cent of all patients taking sulfadiazine (8) and 2.6 per cent of those taking monomethylsulfadiazine. Leukopenia and anemia were not encountered. Delirium was unusually frequent with the methyl homologues whereas in 588 sulfadiazine-treated patients, only 8 instances or 1.3 per cent were observed, and of the 38 dimethylsulfadiazine-treated patients, 12 were in delirium. Three of these became delirious before therapy started and continued so afterwards. Nine patients became delirious during the therapy, a 23.6 per cent incidence. In this series, 3 patients vomited following the ingestion of the drug, and hematuria occurred in 1 patient. In the dimethylsulfadiazine group, 13 of the 64 cases, or 20.3 per cent, became delirious

after the drug was administered. One of the monomethylsulfadiazine-treated patients developed hematuria and anuria and was cystoscoped. Cystoscopy revealed a blocked ureter and large crystal aggregates floating in the urine of the bladder, and embedded in the mucosa of the bladder. The drug was discontinued. The patient was relieved of his anuria by ureteral irrigation and recovered.

Of the 38 patients treated with monomethylsulfadiazine, 4 died. Two of these deaths occurred 10 to 12 hours after commencing the administration of the drug.

The first instance of death 10 hours after commencement of therapy occured in a patient who had a B. Friedlander A infection with a left lower lobe involvement. The drug was started on the second day of his illness and he had received a total of 6 grams prior to the onset of delirium. Following the administration of the drug, he vomited, became delirious, and died in a convulsion. He had received a total of 8 grams.

The second instance of death which occurred 12 hours following the beginning of drug therapy was that of a

TABLE III

Delirious cases and deaths in patients treated with monomethylsulfadiazine

Patient	Day of disease admitted to hospital	Sex	Age	Pneumococcus type	Lobes involved	Day of disease therapy began	Total no. of days drug given	Blood levels: Day of disease	Blood levels: Mgm. per 100 cc.	Initial temp. drop in hours after therapy began	No. of grams of drug given before initial drop in temp.	Delirium: Day of disease of onset	Delirium: Temp. at onset	Delirium: No. of grams of drug given prior to onset	Delirium: Total no. of grams drug given	Delirium: No. of days in delirium	Delirium: Day of disease delirium terminated	Delirium: Day of disease drug stopped	Outcome
			years																
W. H.	4	M	38	IX	RUL RML X-ray	4	6	5 7	10.0 7.0	30	11	5	103°	9	29	4	9	10	Recovered
H. B.	4	M	43	III	RLL	4	7	5 7 8	14.4 19.8 16.4	40	7	6	101.4°	15	37	4	10	10	Recovered
R. F.	6	M	24	I	LLL X-ray	6	4	7 10	11.2 2.0	32	15	8	99°	15	19	2	10	9	Recovered
J. T.	4?	M	38	XII	LLL	4?	2					On admission	103°	0	7	?	?	?	Patient was in shock on admission and died 12 hours after therapy began
W. E.	8	M	35	VII	RUL–RML RLL X-ray	8	8	9 11 13 16	8.6 18.6 14.2 6.8	28	9	9	104.8°	6	39	3	12	12	Recovered
J. N.	?	M	42	XVII	RLL–RML	?	3	? 5/13	6.0			On admission			18				Died in delirium 52 hours after the drug was given. Received 340,000 units of anti-pneumococcic serum.
E. B.	2	M	38	BFA	LLL	2	1	?						6	8				Died in convulsions 10 hours after therapy was started. This patient vomited.
N. T.	?	M	72	VIII	LLL–RLL	?	2	? 6/5	2.8			On admission			8				Died 28 hours following drug therapy.
J. W.	4	M	28		RLL–X-ray	4	4	6 7 9	19.8 11.0 7.8	44	11	8	99°	23	23	2	10	7	Recovered
W. S.	?	M	32	VI	RLL–LLL	?	4	? 5/22 5/24 5/25	5.6 17.6 18.0	76	19	? 1	101°	14	23	2	3?	5?	Recovered
V. C.	2	F	46		RML–RLL X-ray	2	3	4	18.0			6	102°	18	18	2	8	6	On the 6th day of the disease, monomethylsulfadiazine was discontinued. The patient was in delirium. On the 7th day of illness sulfathiazole was administered. Patient recovered.
G. T.	2	M	32	XI	LLL	2	4	3 5	9.4 19.8	48	5	4	101°	10	16	2	6	5	Recovered

All patients received monomethylsulfadiazine, 4 grams *statim* and 1 gram every 4 hours day and night.

TABLE IV

Comparative mortalities with sulfadiazine, monomethylsulfadiazine, and dimethylsulfadiazine

	Sulfadiazine			Monomethyl-sulfadiazine			Dimethyl-sulfadiazine		
	Cases	Deaths	Per cent	Cases	Deaths	Per cent	Cases	Deaths	Per cent
Total	232	31	13.4	38	4	10.3	64	10	15.8
Total minus deaths within 24 hours after therapy began	219	18	8.2	36	2	5.5	61	7	11.4

patient suffering from a pneumococcus Type XII pneumonia involving the left lower lobe. The drug was started on the fourth day of his disease and he had received a total of 7 grams. He was delirious on admission and continued in delirium after the drug was administered and without modification of his condition otherwise.

A third death occurred 52 hours after the drug was started. The patient was suffering from a Type XVII pneumococcus pneumonia with right lower lobe and right middle lobe involvement. The drug was administered on admission and he received a total of 18 grams in 3 days. He was admitted in delirium from which he did not recover. Additional therapy included 340,000 units of the pneumococcus XVII antiserum.

The fourth death occurred 28 hours after therapy was instituted. X-ray revealed left lower lobe and right lower lobe involvement. He received 8 grams in 2 days. He was delirious on admission and continued so after the administration of the drug.

In addition to the 4 fatal cases, there were 8 instances of delirium. The time of onset of delirium following the administration of the drug varied from 1 to 4 days. Some deliria were mild. The patients were out of contact as shown by hallucinations and irrelevancy. Other delirious patients were active and required restraints.

In 1 patient, aged 43, delirium started on the 6th day of his illness, 46 hours after the drug was given. He had received 15 grams at the time of onset of the delirium. This patient recovered from a pneumococcus III with right lower lobe involvement. He received a total of 37 grams in 7 days. The initial temperature drop occurred 40 hours after the drug was started.

In another patient, aged 35, delirium started on the 9th day of his disease. He had received 6 grams of monomethylsulfadiazine at the time of onset of delirium. This patient recovered from a pneumococcus VII pneumonia. X-ray revealed right upper lobe, right middle lobe, and right lower lobe involvement. He received a total of 39 grams in 8 days. The initial temperature drop occurred following the ingestion of 9 grams of the drug (28 hours). Hiccoughs occurred soon after the drug was given and were not relieved by oxygen-carbon dioxide inhalation.

In a 3rd patient, aged 24, delirium occurred on the 8th day of his illness after 15 grams of monomethylsulfadiazine were administered. The drug was started on the 6th day of his disease. This patient recovered. He had a pneumococcus Type I pneumonia. X-ray showed left lower lobe involvement. He received a total of 19 grams in 4 days. The initial temperature drop started after he had received 15 grams of the drug.

A 4th patient, aged 38, became delirious on the 5th day of his illness, 26 hours after the drug was started. He received 9 grams prior to the onset of delirium. This patient recovered from a pneumococcus Type IX pneumonia. X-ray showed right upper lobe and right middle lobe involvement. He had received a total of 29 grams in 6 days. The initial fall in temperature occurred after he received 11 grams of the drug.

Other instances of delirium are indicated in Table III.

Table IV offers the mortality comparison of the drugs studied. In 232 cases treated with sulfadiazine, there were 31 deaths or a mortality of 13.4 per cent. In this series, 13 deaths occurred within 24 hours after therapy began, making the mortality after 24 hours of therapy 8.2 per cent. Four out of the 38 cases treated with monomethylsulfadiazine, or 10.5 per cent, died. In this series, 2 deaths occurred within 24 hours after therapy started, with a mortality of 5.5 per cent after 24 hours of therapy. Of the 64 patients treated with dimethylsulfadiazine, 10 patients, or 15.6 per cent, died. In this group, 3 cases died within 24 hours following therapy, a mortality of 11.4 per cent.

Sulfadiazine, when effective, will generally cause a drop in temperature in about 24 hours following its administration. With monomethylsulfadiazine, we observed 28 cases that followed this trend while 10 patients required more than 24 hours before the temperature fell.

SUMMARY

Monomethylsulfadiazine and dimethylsulfadiazine, like sulfadiazine, are readily absorbed from the gastrointestinal tract. Blood concentrations, when equal doses are given, are generally higher for monomethylsulfadiazine than for sulfadiazine. Rapid attainment and maintenance of high blood levels may be accomplished with monomethylsulfadiazine. In the urine, about 35 per cent of the amount of sulfadiazine recovered was conjugated; of monomethylsulfadiazine, 50 per cent; and of dimethylsulfadiazine,

45 per cent. The incidence of mental disturbances with monomethylsulfadiazine was 23.6 per cent; with dimethylsulfadiazine, 20.3 per cent; and with sulfadiazine, 1.3 per cent. Gross hematuria was observed with sulfadiazine and monomethylsulfadiazine. Polyuria was noted with dimethylsulfadiazine. The mortality in cases of pneumonia treated with the 3 drugs was,—sulfadiazine 8.2 per cent in 232 cases; monomethylsulfadiazine, 5.5 per cent in 38 cases; for dimethylsulfadiazine, 11.4 per cent in 64 cases.

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DEPRESSANT EFFECTS OF HIGH CONCENTRATIONS OF INSPIRED OXYGEN ON ERYTHROCYTOGENESIS. OBSERVATIONS ON PATIENTS WITH SICKLE CELL ANEMIA WITH A DESCRIPTION OF THE OBSERVED TOXIC MANIFESTATIONS OF OXYGEN [1,2]

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This report describes the effect of the continuous inhalation of 70 to 100 per cent oxygen for 8 to 20 days on the cellular elements of the blood of patients with sickle cell anemia. The investigation was originally begun in an effort to reduce the degree of intravascular sickling of red blood cells. There were two reasons for trying to produce this result: (*1*) Diggs (1) and Bauer (2) have suggested that the pain of sickle cell crises may result from anoxia of tissues caused by occlusion of blood vessels with elongated clumped cells, and (*2*) the increased rate of hemolysis in sickle cell anemia might possibly be due to the greater trauma to which sickled cells are subjected during circulation. Therefore, if the abnormal shape could be corrected, the pain of crises might be relieved, the rate of hemolysis decreased, and the degree of anemia lessened. It is well known that *in vitro* the erythrocytes of patients with sickle cell anemia will become biconcave discs when they are exposed to air or oxygen, and become sickled when they are exposed to carbon dioxide (3, 4). Sherman (4), furthermore, demonstrated that there are fewer sickled cells in arterial than in venous blood. But, when this study was begun, no one had shown that the oxygen tension of arterial blood could be increased sufficiently to influence the degree of intravascular sickling. That this result might be accomplished, however, seemed likely since (*1*) Boothby, Lovelace, and Uihlein (5) were able to increase alveolar oxygen tension to more than 500 mm. Hg, and (*2*) Behnke (6) had shown that the oxygen tension of arterial blood approximates that in the alveoli.

Oxygen was administered for periods of 8 to 20 days on 6 different occasions to 4 patients with sickle cell anemia. Early in the study it became obvious that the degree of intravascular sickling was diminished during the periods of oxygen inhalation, but there was no evidence that the rate of hemolysis or the occurrence of pain was affected. However, there were definite indications that erythrocytogenesis was depressed. On the fourth to sixth day of oxygen administration, the reticulocyte level began to fall, and several days later the red blood cells also began to decrease in number. During 3 of the 6 periods, the red cell counts fell by more than 1,000,000 cells. Four or 5 days after the administration of oxygen was discontinued, a pronounced reticulocytosis developed and the number of red cells returned to approximately the pre-oxygen level. Congestion of the mucous membrane of the upper respiratory passages, anorexia, and nausea were the principal evidences of toxic effects produced by the high concentrations of oxygen.

I. Methods and experimental conditions

Red and white blood cell counts were made with pipettes and hemocytometer chambers that had been standardized by the United States Bureau of Standards. Hemoglobin determinations were made by the oxyhemoglobin method of Evelyn (7). Differential leukocyte counts were made on supravital preparations. Platelets were estimated by Dameshek's technic (8). Both wet and dry preparations were used for counting reticulocytes. Wet and fixed films were used in applying Sherman's method for the determination of the percentage of sickled cells (4).

Changes in the rate of hemolysis were estimated from determinations of serum bilirubin, serum iron, and the excretion of urobilinogen and stercobilinogen. Quantita-

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[2] Presented in part at the 15th Annual Meeting of the Central Society for Clinical Research, Chicago, November 6, 1942.

tive estimation of the serum bilirubin was made with an Evelyn photoelectric colorimeter (9). Serum iron values were obtained with the thiocyanate technic (10). The method of Watson was used for measuring the excretion of urobilinogen and stercobilinogen (11). Urine and stool specimens were collected in 4-day periods.

The oxygen content of arterial and venous blood was determined on 1 ml. of blood by the method of Van Slyke and Neill (12a) as described in Peters' and Van Slyke's Quantitative Clinical Chemistry, Methods (12b). The CO_2 and O_2 were liberated from the blood by vacuum extraction with gas-free saponin-ferricyanide-lactic acid mixture and the pressure of the combined gases was read at 2 ml. volume in Van Slyke's manometric gas analysis apparatus; the CO_2 was absorbed from the mixture with air-free and carbonate-free N NaOH and the pressure was again taken at 2 ml.; the oxygen was absorbed with freshly prepared alkaline hyposulfite solution and the pressure was again read at 2 ml. volume; finally the pressure of the residual nitrogen was read at 0.5 ml. volume. Several times during the study, alveolar air samples were collected by the method of Henderson and Morriss (13) and were analyzed for partial pressures of oxygen, nitrogen, and carbon dioxide (technic of Van Slyke and Sendroy) (14).

Oxygen was administered by means of a Boothby-Lovelace-Bulbulian (B.L.B.) mask (5). A nasal mask was worn continuously during the day, even at meal-times. At night, the oronasal mask was substituted so that patients who opened their mouths during sleep would not breath air. One of the subjects (B.W.) did not tolerate the face mask well; she was permitted to wear the nasal mask day and night. Repeated observations revealed that she did not sleep with her mouth open. In all cases, the oxygen flow was kept at 7 to 8 liters per minute, and a humidifier was used. Control periods before and after the periods of oxygen administration varied from 4 days to a month or more.

Case reports describing the patients studied in this investigation are recorded at the end of the paper.

II. *Concentrations of alveolar oxygen obtained; degree of saturation of arterial and venous blood*

Boothby has shown that it is possible to maintain the alveolar oxygen at about 90 to 92 per cent with the B.L.B. inhalation apparatus when the rate of flow of oxygen is 7 to 8 liters per minute (5). This, he showed, corresponds to approximately 100 per cent oxygen in the inspired air. He also found that when the nasal mask is worn and the patient is permitted to eat or talk, the alveolar oxygen content falls to 65 per cent. The 4 determinations of alveolar oxygen content made in the present study during periods of oxygen administration gave values which varied from 63.4 to 90.6 per cent oxygen (Table I). These figures are roughly equivalent to levels of 70 to 100 per cent oxygen in the inspired gas mixture (15). The alveolar oxygen content unquestionably fell at meal-times and when the subjects talked; it is unlikely, however, that the percentage was often lower than 60 during the periods of oxygen administration.

TABLE I

Percentages of O_2, N_2, and CO_2 in alveolar air collected during periods of oxygen therapy

Patient	Date	Oxygen therapy or control	Alveolar gas		
			O_2	N_2	CO_2
			volumes per cent		
B. W.	7/17/42	7th day of O_2 therapy	63.4	31.4	5.3
	7/28/42	18th day of O_2 therapy	90.6	5.9	3.6
	7/29/42	19th day of O_2 therapy	81.0	13.6	5.7
	8/17/42	17th day after O_2 was discontinued	16.8	78.6	4.5
T. S.	1/19/43	12th day of O_2 therapy	77.5	18.0	4.2

Further evidence of the high oxygen tension produced in alveolar air was provided by the increased oxygen saturation observed in arterial and venous blood. Both the combined and dissolved oxygen of blood are dependent on the partial pressure of oxygen in the alveoli. Boothby has shown that the amount of oxygen in 100 ml. of arterial blood of the average normal individual can be increased from 19.5 to 22.2 ml. when 100 per cent oxygen is inhaled instead of air (16). This amounts to an increase of 10 to 15 per cent in the oxygen content of arterial blood and produces a definite change in the partial pressure of oxygen in both the capillaries and tissues (16). The results obtained in these 4 patients were similar to those reported by Boothby. Determinations of oxygen content and capacity of both arterial and venous blood were usually made at 4-day intervals. With few exceptions, while pure oxygen was breathed, the oxygen content of arterial blood was greater than the so-called "oxygen capacity" determined with room air and the oxygen saturation of venous blood was invariably raised. Data obtained on one of the patients and illustrative of the changes observed are recorded in Table II.

TABLE II

Oxygen content, capacity, and saturation of venous and arterial blood of patient before, during, and after oxygen administration

Patient L. McC., colored female, age 25 years

Date	Arterial			Venous		
	Content	"Capacity"	Sat.	Content	"Capacity"	Sat.
			per cent			*per cent*
3/ 9/42	7.9	8.7	90.8	3.1	8.6	36.0
3/18/42	9.2	10.3	89.3	3.1	10.3	30.1
3/18/42			oxygen started			
3/22/42	10.1	10.0	101.0	6.3	10.0	63.0
3/30/42	8.4	8.0	105.0	3.9	7.9	49.4
3/30/42			oxygen stopped			
4/3/42	5.7	6.9	82.6	1.4	6.9	20.3
4/ 8/42	7.5	10.1	74.2	2.9	10.1	28.7
4/20/42	12.0	14.2	84.5	5.1	14.2	35.9

Determinations made on 1 ml. samples of blood according to method described by Peters and Van Slyke (12b).

III. *Changes in the degree of sickling produced by oxygen administration*

When the percentage of sickled cells was estimated in samples of arterial and venous blood, considerable difficulty was experienced in deciding which cells should be called "sickled." The difficulty can easily be understood because, as Diggs has demonstrated, the abnormal cells show all degrees of sickling (17). There were, in the preparations made by Sherman's technic (4), many oblong and irregularly shaped red cells which were not truly sickled. Cells of this type are labeled "Ab" in Figure 1, while frankly sickled cells are marked by the letter "S." In order to compensate for some of this difficulty, counts during the last 4 periods of this study were done of both (*a*) the total abnormal cells, and (*b*) the true sickled cells. It was not possible to establish sharply defined rules governing each of these categories, and frequently there was considerable variation in the counts done by two different observers. The figures tabulated in Table III represent the average of values obtained each time by the same two persons when several thousand cells were counted.

From the data in Table III, it can be seen that there were more abnormal forms in venous than in arterial blood. This result is consistent with the fact that the oxygen tension of arterial blood is greater than that of venous blood. In most instances, during the time of oxygen administration, the percentage of abnormal forms was less in both arterial and venous blood than during pre- and post-oxygen control periods, a finding which confirms the observation made by Klinefelter (18). The most definite change occurred when oxygen inhalation was discontinued; the percentage of sickled cells rose sharply and then gradually declined until basal levels for each subject were reached.

IV. *Rate of hemolysis as influenced by oxygen administration*

Whenever the rate of hemolysis is accelerated, the excretion of urobilinogen and of stercobilinogen is increased, the icterus index and serum bilirubin become elevated, and the serum iron value rises (if erythrocytogenesis is unaltered) (19). These changes are reversed when the rate of hemolysis slows. Watson has presented evidence to show that variations in the excretion of urobilinogen and stercobilinogen constitute the

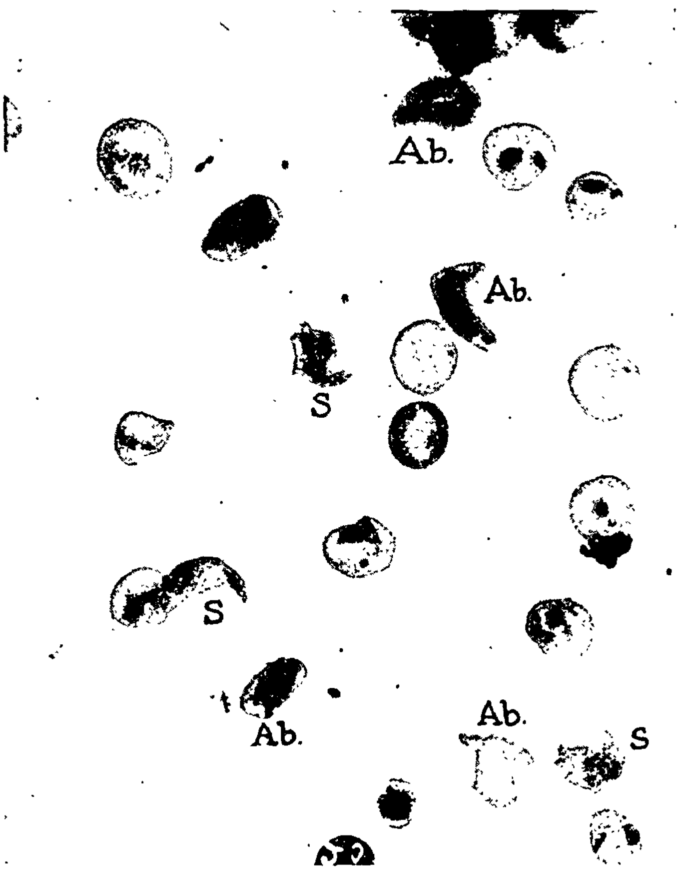


FIG. 1. FORMALIN FIXED SICKLE CELL PREPARATION

In counting such a preparation, cells marked S were recorded as grossly sickled while cells marked Ab. were recorded as abnormal.

most sensitive known index of the rate of red blood cell destruction (20). Results pertaining to this excretion will, therefore, be presented first. In order to avoid unnecessary repetition, the term "urobilinogen excretion" will be used to designate excretion of both urobilinogen and stercobilinogen; both chromogens are determined by the method used.

Data on the excretion of urobilinogen of these 4 subjects are presented graphically in Figures 2 to 6. From these graphs, it is apparent that no consistent change was produced in the excretion of urobilinogen produced by oxygen administration. Furthermore, when the values for urobilinogen excretion were averaged for all oxygen and all control periods, the average figures again failed to demonstrate any consistent effect of oxygen administration on the excretion of urobilinogen (Table IV).

TABLE III

Percentage of abnormal erythrocytes in arterial and venous blood before, during, and after periods of oxygen administration

Patient	Date	Arterial blood		Venous blood	
		Total abnormal count	True sickled count	Total abnormal count	True sickled count
		per cent	*per cent*	*per cent*	*per cent*
G. C.	12/ 5/41	14		54	
	12/ 8/41	13		30	
	12/12/41	24		44	
	12/12/41	oxygen started			
	12/17/41	7		27	
	12/19/41	15		30	
	12/20/41	23		30	
	12/20/41	oxygen stopped			
	12/24/41	35		49	
B. W.	12/30/41	19		34	
	12/30/41	oxygen started			
	1/ 3/42	14		17	
	1/ 7/42	21		26	
	1/ 7/42	oxygen stopped			
	1/11/42	41		50	
L. McC.	3/ 9/42	44	4	47	22
	3/18/42	oxygen started			
	3/22/42	20	5	44	17
	3/26/42	32	8	34	19
	3/30/42	33	6	48	29
	3/30/42	oxygen stopped			
	4/ 3/42	45	16	67	37
	4/ 8/42	37	10	52	30
L. McC.	4/20/42	38	12	61	39
	4/27/42	42	16	65	41
	4/27/42	oxygen started			
	5/ 1/42	25	8	46	19
	5/ 5/42	32	7	43	25
	5/ 9/42	oxygen stopped			
	5/13/42	37	12	47	29
B. W.	7/10/42	38	9	51	23
	7/10/42	oxygen started			
	7/14/42	37	2	47	15
	7/18/42	35	3	41	20
	7/23/42	39	2	56	15
	7/27/42	34	2	61	17
	7/30/42	oxygen stopped			
	8/ 4/42	54	5	67	27
	8/ 7/42	48	4	76	30
	8/14/42	34	2	44	18

TABLE IV

Average total excretion of urobilinogen in urine and stool

Patient	Control periods	Oxygen periods
	mgm. per 24 hours	
G. C.	261	139
B. W.	755	778
L. McC.	346	331

Values for the icterus index, serum bilirubin, and serum iron showed fluctuations which were apparently entirely independent of the oxygen administration. The data are summarized in Table V.

No evidence was obtained, therefore, with these methods of study to indicate that the administration of high concentrations of oxygen to patients with sickle cell anemia influences the rate at which red blood cells are destroyed.

V. *Changes in erythrocytogenesis produced by oxygen administration*

During each of the 6 periods of oxygen administration, there were evidences of a striking decrease in the rate of erythrocytogenesis. The initial red blood cell counts in all 4 patients varied from 2 to 3 million cells per cu. mm. This anemia was unquestionably a reflection of the rapidity with which red cells were being destroyed. That the bone marrow was stimulated and trying to compensate for the hemolysis was indicated by the facts that (*1*) a reticulocytosis of 15 to 30 per cent was present in each case and (*2*) there was a great predominance of normoblasts in the bone marrow preparations studied. The first change observed in the peripheral blood after a subject began breathing pure oxygen was a decline in reticulocytes which began on the fourth or fifth day of oxygen therapy. Within 48 to 72 hours, the reticulocyte percentage had decreased to 2 to 4 and the number of erythrocytes

TABLE V

Effect of oxygen administration on the icteric index, serum bilirubin, and serum iron

Patient	Date	Icterus index	Serum bilirubin		Serum iron
			Direct	Indirect	
			mgm. per cent	*mgm. per cent*	*mgm. per cent*
B. W.	12/29/41		0.84	2.42	
	12/30/41		oxygen started		
	1/ 5/42		0.63	1.54	
	1/ 7/42			1.24	
	1/ 7/42		oxygen stopped		
	1/11/42			1.34	
L. McC.	3/10/42		1.32	1.67	0.102
	3/14/42	6	0.24	0.43	0.041
	3/18/42		oxygen started		
	3/23/42	7	0.42	0.79	0.092
	3/26/42	13	0.63	1.38	0.177
	3/30/42		oxygen stopped		
	4/ 2/42	15	0.63	1.40	0.215
	4/ 8/42	25	0.94	1.68	0.170
	4/20/42	30	0.73	2.17	0.201
	4/27/42	20	0.70	1.77	0.101
	4/27/42		oxygen started		
	5/ 1/42				0.064
	5/ 5/42	13	0.61	1.10	
	5/ 9/42		oxygen stopped		
B. W.	7/ 6/42	10			0.092
	7/10/42	10			0.133
	7/10/42		oxygen started		
	7/14/42	15			0.071
	7/18/42	7			0.146
	7/23/42	5	0.61	1.13	0.061
	7/27/42	5			0.097
	7/30/42	16			0.190
	7/30/42		oxygen stopped		
	8/ 4/42	7	0.82	1.90	0.133
	8/ 7/42	10	0.68	1.44	0.153
	8/14/42	5	0.75	1.78	0.115

had also begun to fall. The drop in number of red blood cells was occasionally as much as one million cells or more. These relationships are graphically illustrated in Figures 2 to 6. The reticulocytes remained at their low levels as long as oxygen administration was continued except in one patient during a period which lasted for 20 days (Figure 4). During the last 10 days of this period, the patient complained bitterly about the discomfort caused by her mask and was found repeatedly with the mask removed. Her reticulocyte count began to rise on the fifteenth day and reached a level of 17 per cent at the time oxygen was stopped. No change occurred in the percentage of reticulocytes during the other 5 periods until about 48 hours after oxygen was discontinued. The reticulocyte level then began to rise rapidly, reached a peak on the seventh or eighth day, remained elevated for 5 to 12 days, and then fell to approximately the pre-oxygen level. The post-oxygen peak reticulocyte values were surprisingly high and varied from 53 to 82 per cent. Shortly after the reticulocyte percentage began to rise, the red blood cells, hemoglobin, and hematocrit levels also started to increase. Within several weeks, their initial values had been regained, and in several instances had even become higher than the pre-oxygen levels.

Coincident with the reticulocytosis which followed the cessation of oxygen therapy, a shower of nucleated red blood cells also appeared in the peripheral blood. Each one of the 4 patients showed an occasional normoblast in the blood under basal conditions, but the number increased sharply on the third day after oxygen, reached a peak on the sixth or seventh day, and then fell to "basal" levels within 3 or four more days. In one instance (Figure 5), when this development was most marked, 2.1 per cent of all the red blood cells in the peripheral circulation were normoblasts.

The progression of changes brought about by the oxygen administration (fall in reticulocytes followed by a decrease in erythrocytes and hemoglobin) was interpreted as evidence of a striking inhibition of erythrocytogenesis. It is suggested that this result is the physiological antithesis of the stimulation of red cell formation which occurs at low oxygen tensions. This interpretation is supported by the tremendous increase in erythrocytogenesis which followed cessation of oxygen administration with consequent lowering of alveolar and arterial oxygen tensions. It is believed that the one subject who developed an increase in reticulocytes while she was still breathing oxygen did so because she removed her mask often enough to lower her alveolar oxygen content, and partially release her marrow from the inhibition of the high oxygen tensions. An alternative explanation might be that her bone marrow became acclimated to the high oxygen tension and began to "escape" the inhibitory effect. In animal experiments, it has been found that, with long continued residence in 95 per cent oxygen chambers, the depression of bone marrow may or may not be continuous; if not, the reticulocytes increase (21).

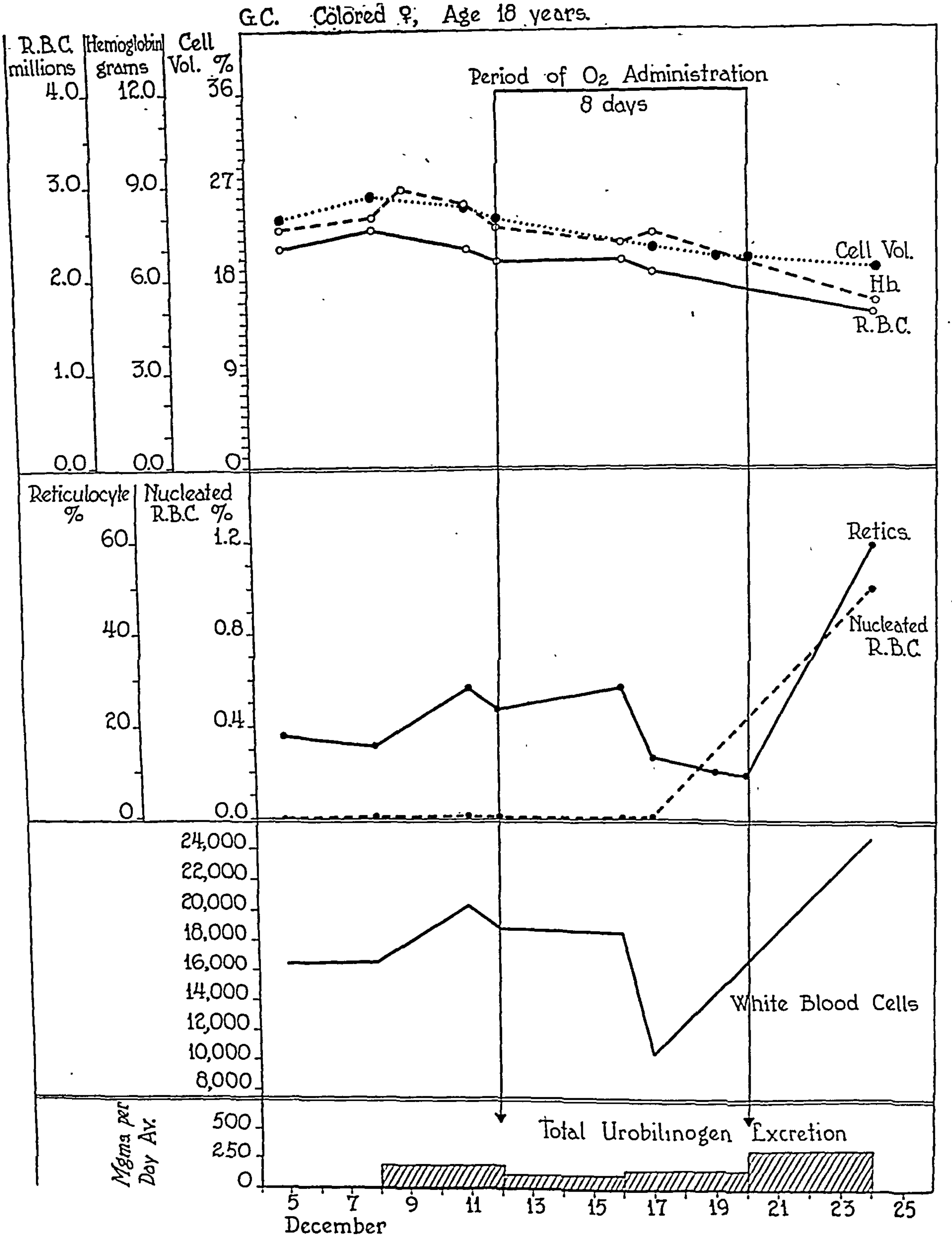


FIG. 2. EFFECT OF ADMINISTERING PURE OXYGEN (B.L.B. MASK) ON THE ERYTHROID EQUILIBRIUM OF A PATIENT WITH SICKLE CELL ANEMIA

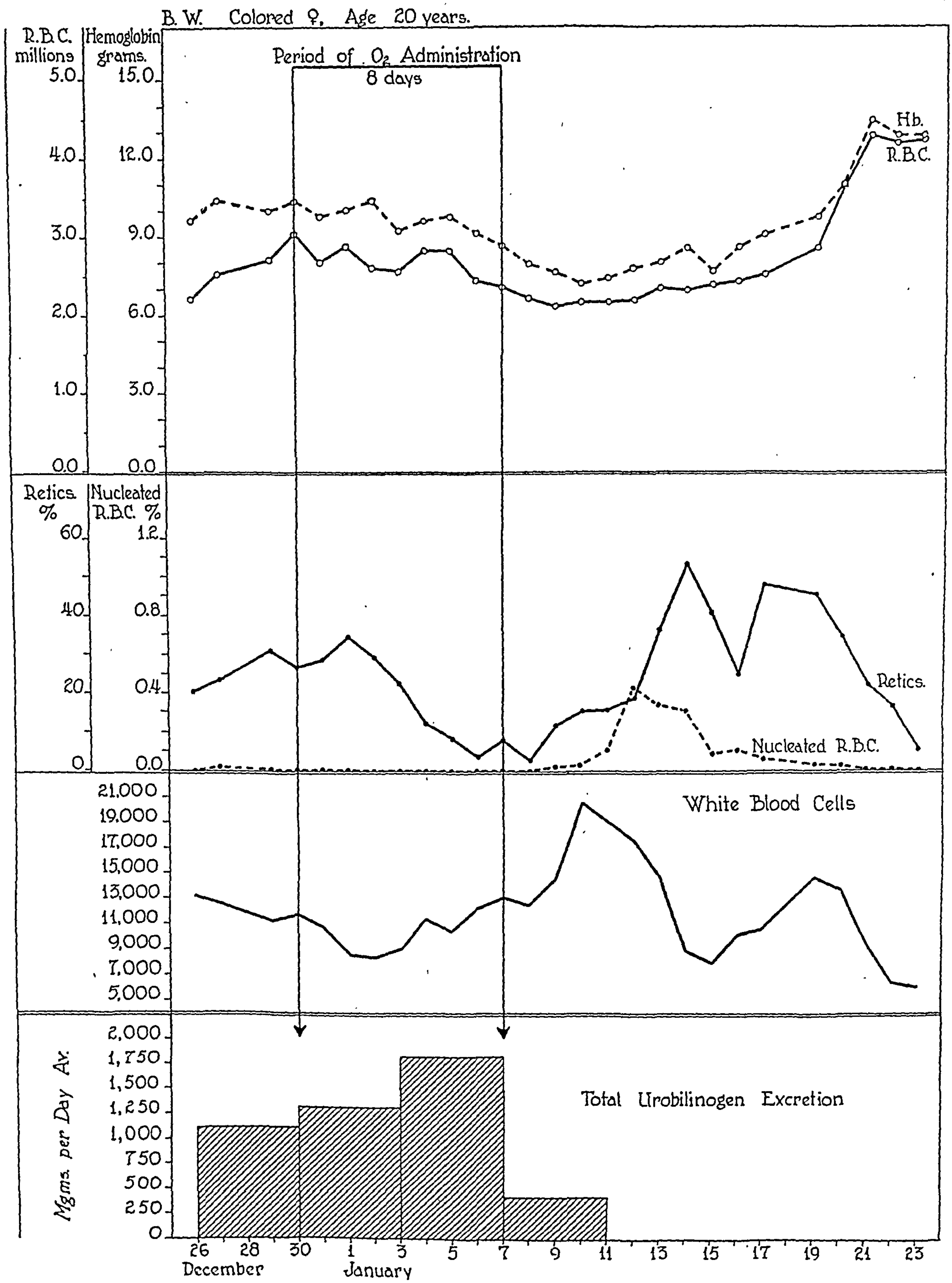


FIG. 3. EFFECT OF ADMINISTERING PURE OXYGEN (B.L.B. MASK) ON THE ERYTHROID EQUILIBRIUM OF A PATIENT WITH SICKLE CELL ANEMIA

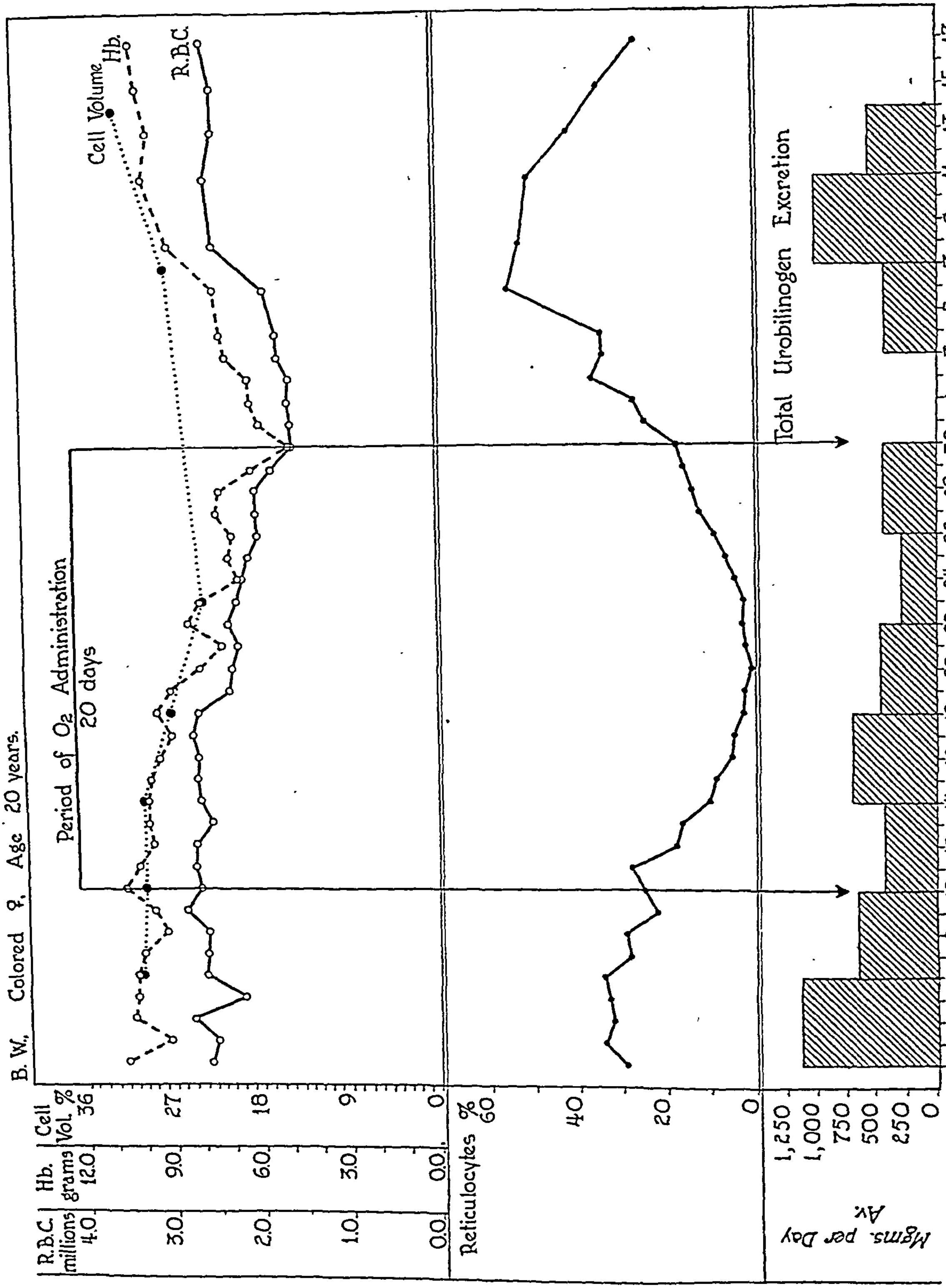


FIG. 4. EFFECT OF ADMINISTERING PURE OXYGEN (B.L.B. MASK) ON THE ERYTHROID EQUILIBRIUM OF A PATIENT WITH SICKLE CELL ANEMIA

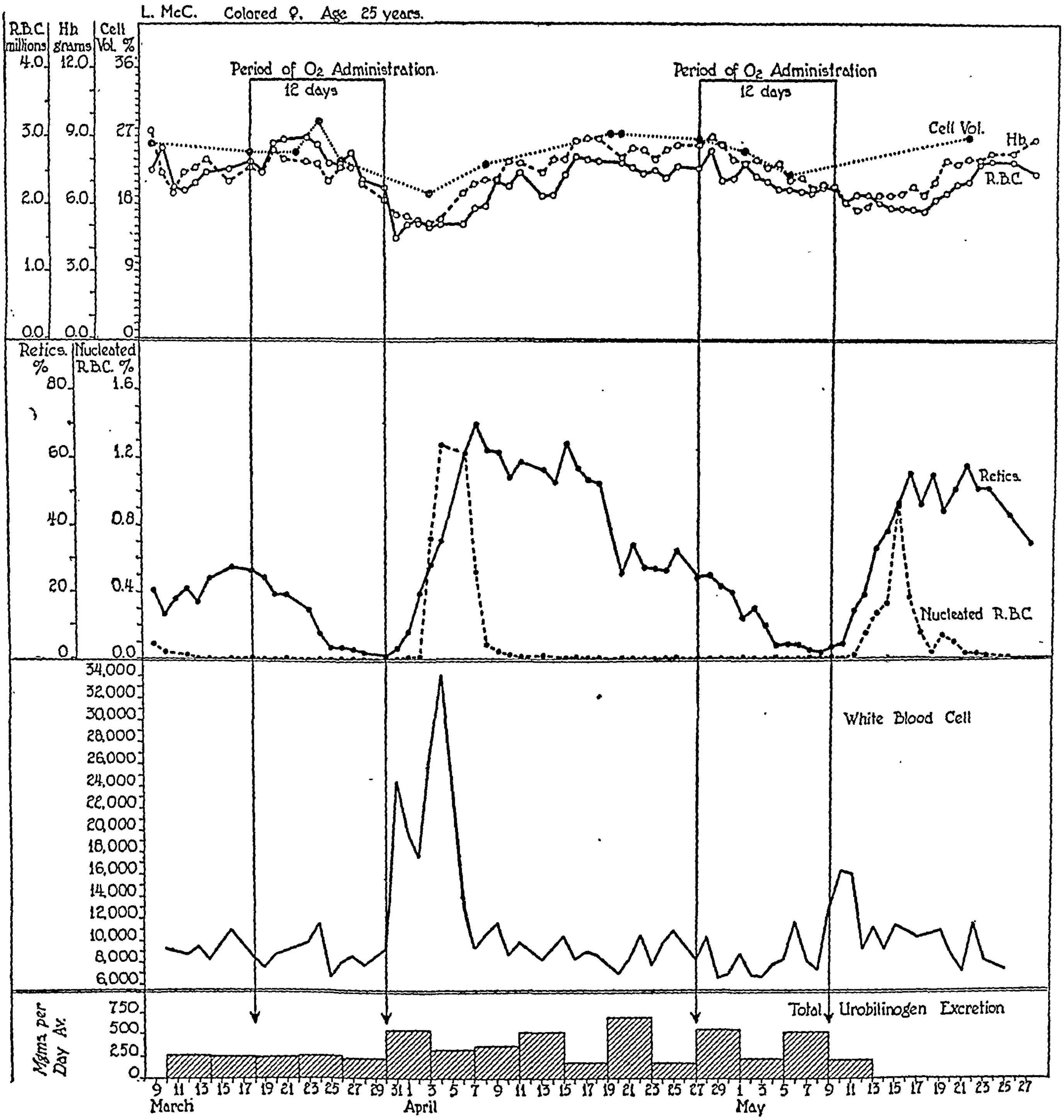
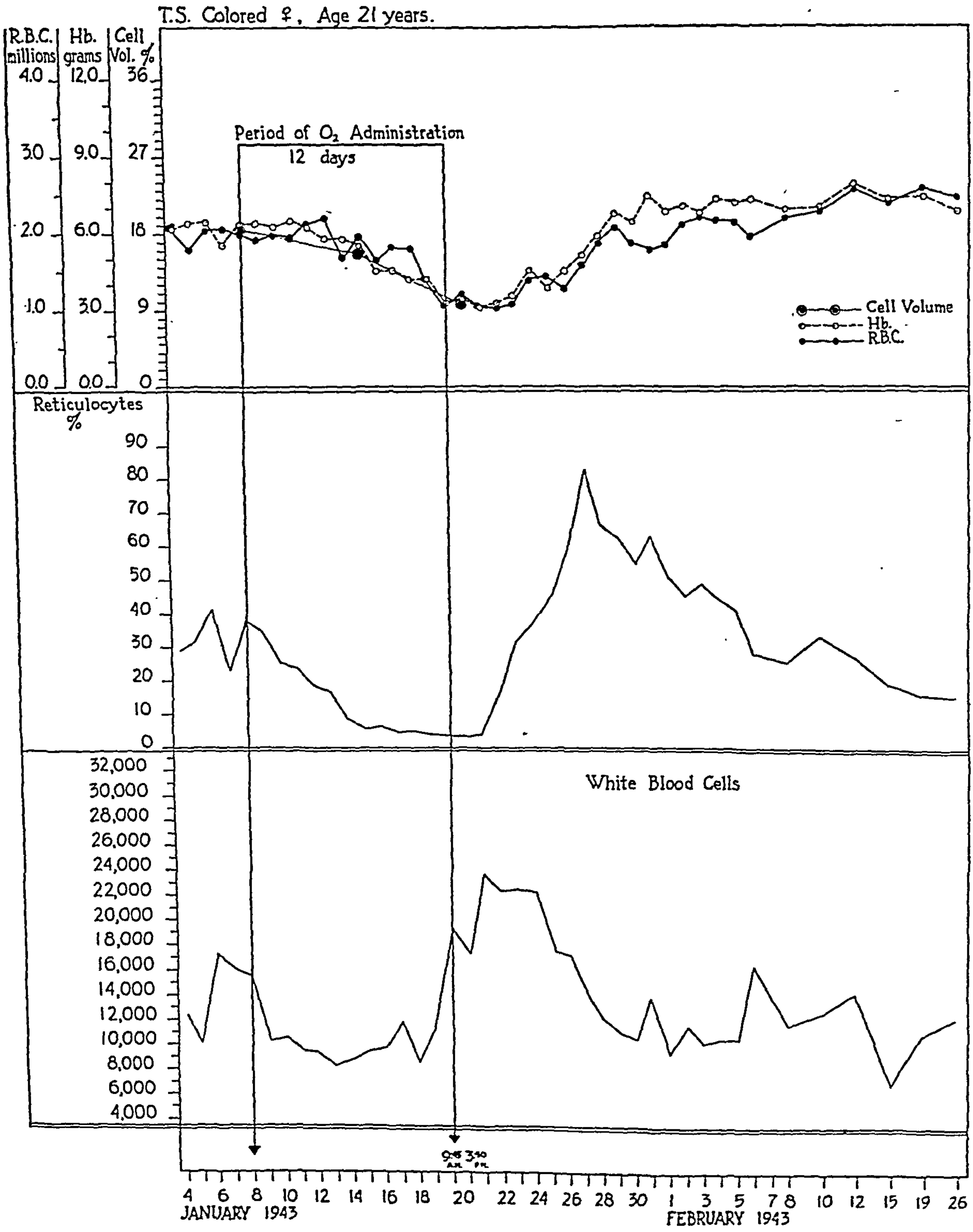


FIG. 5. EFFECT OF ADMINISTERING PURE OXYGEN (B.L.B. MASK) ON THE ERYTHROID EQUILIBRIUM OF A PATIENT WITH SICKLE CELL ANEMIA



T.S. Colored ♀, Age 21 years.
R.B.C. millions
Hb. grams
Cell Vol. %
Period of O_2 Administration
12 days
Cell Volume
Hb.
R.B.C.
Reticulocytes %
White Blood Cells
9:45 A.M.
3:30 P.M.
JANUARY 1943
FEBRUARY 1943

FIG. 6

VI. *Changes in the leukocytes and platelets produced by oxygen administration*

Total white blood cell counts, corrected for the nucleated red cells present, did not change significantly during the periods of oxygen inhalation. In every instance, however, a significant increase in the number of leukocytes developed within 24 to 48 hours after oxygen was discontinued and lasted for a period of 4 to 5 days. This leukocytosis was caused primarily by an absolute increase of mature polymorphonuclear neutrophiles. The number of neutrophilic leukocytes was frequently 2 to 3 times as great as it had been during the period of oxygen therapy. Myelocytes, metamyelocytes, and non-segmented neutrophiles also were increased, an occurrence which suggested that the leukocytosis was caused by an outpouring of cells from the bone marrow rather than by a redistribution phenomenon. Basophilic leukocytes, lymphocytes, and monocytes also participated in the leukocytosis but to a lesser degree than did neutrophilic elements.

No significant changes occurred in the platelet counts during or after oxygen administration.

VII. *Evidences of the toxicity produced by pure oxygen*

As was stated earlier, the actual concentration of oxygen in the inspired gas mixture varied, in all probability, from about 70 to 100 per cent, depending upon the activity of the subject. Since these concentrations of oxygen have been found to produce "oxygen pneumonia" and pulmonary edema in animals, the patients were examined very frequently and occasional roentgenograms of the chest were made. No objective evidence of pulmonary congestion or inflammation was detected although one patient did develop a cough. On 2 occasions, oxygen inhalation was accompanied by evidences of irritation of the mucous membrane of the upper respiratory tract (nasal congestion, lacrimation, sore throat, and hoarseness), but in both instances, these symptoms disappeared before oxygen administration was discontinued. Epistaxis occurred twice. Three of the 4 subjects developed anorexia; emesis became distressing in one patient and developed in another after the oxygen inhalation had been stopped. Headache was moderately persistent and annoying in 2 subjects. Paresthesias of the hands or feet were present in 2 of the patients, tenderness of calf muscles in one, and sharp shooting pains in the arms and shoulders in another. There were no signs of nervousness or mental instability. Since these manifestations have considerable clinical interest, a detailed statement of the symptoms experienced by each subject is included with the case histories at the end of this paper.

These symptoms are mild as compared with those shown by animals who are exposed to atmospheres high in oxygen. Adams found that guinea pigs died in 4 days from a sterile pneumonia when they were made to breathe 70 to 100 per cent oxygen (22). Barach has reported that pulmonary edema invariably develops in rabbits given oxygen in concentrations of 80 per cent or more (23). Binger also observed pneumonia in dogs, rabbits, and guinea pigs exposed to 80 per cent oxygen; the first symptoms of respiratory distress appeared in 2 to 5 days (24). Paine, Lynn, and Keys found hyperemia and congestion of the kidneys, liver, and lungs of dogs kept in an atmosphere of greater than 75 per cent oxygen; the lungs showed pulmonary edema in addition to hyperemia. When the oxygen was increased to 95 or 100 per cent, death occurred, on the average, after 39 hours (25). When these workers kept the dogs in oxygen chambers for only a part of each day, however, the animals were able to tolerate a much longer total exposure to the high oxygen atmosphere. Evans quotes Sayers as saying that he subjected rabbits, guinea pigs, and white rats to practically 100 per cent oxygen for 16 hours each day for 50 consecutive days without observing any obvious harmful effects (26).

Other observers, have found, as did we, that the toxic effects of high concentrations of oxygen in the inspired air are usually not so dramatic in man as in animals. Evans administered pure oxygen by face mask to more than 100 cyanotic patients for periods of from 1 to 27 days (26). He states that some patients who had pulmonary edema before oxygen administration was started were greatly benefited by the inhalation of "pure oxygen," as were also patients with acute and chronic bronchitis. Becker-Freyseng and Clamann (27) placed themselves for 65 hours in a

chamber the air supply of which contained 90 per cent oxygen. After 24 hours, nervous symptoms appeared. They developed formication of the fingers and toes, and paroxysmal tachycardia. One of them had attacks of vomiting after about 60 hours. Severe bronchitis with fever developed in one of the subjects and his vital capacity fell from 4000 to 2700 ml. This decrease in vital capacity was interpreted as indicating inflammatory changes in the pulmonary parenchyma. Behnke, Johnson, Poppen, and Motley have also reported nervous and mental symptoms in human subjects who were breathing 96 to 99 per cent oxygen at pressures of 1, 2, 3, and 4 atmospheres (28).

VIII. *Effect of oxygen administration on the pain of sickle cell crisis*

Only one of the patients, G. C., had the severe pain of a sickle cell crisis at the time oxygen inhalation was begun. She had been having pain in both ankles, the left thigh, and the right shoulder for 3 weeks, but these pains had begun to subside on the morning oxygen administration was started. Twelve hours after she began breathing oxygen, she became free of pain and remained so throughout the 8-day period. Pain reappeared within 24 hours after the B.L.B. mask was removed and became severe 3 days later. This time reinstitution of oxygen therapy did not relieve her symptoms. It seems unlikely that the initial disappearance of pain had any causal relationship to the institution of oxygen therapy, but no general conclusions may be drawn from this one experience.

IX. *Discussion*

When high concentrations of oxygen are breathed by patients with sickle cell anemia for 8 to 20 days, the degree of intravascular sickling is decreased, the rate of hemolysis is apparently unaffected, and erythrocytogenesis is depressed. This last observation is the most interesting; it suggests that high tensions of oxygen depress the bone marrow just as low tensions stimulate it. The rôle of oxygen as a principal regulator of red cell regeneration, therefore, is re-emphasized.

Comparatively little attention has been directed in the past to the effect of high oxygen concentrations on bone marrow activity. Bornstein (29) cites experiments by Regnard in which dogs, kept for days in pure oxygen, were found to show a decrease in hemoglobin and red blood cells. Campbell found that a similar decrease in red blood cells and hemoglobin occurred when monkeys, rabbits, rats, guinea pigs, and mice were exposed to 60 per cent oxygen at normal barometric pressure (30, 31); cats did not show the change. The blood elements returned to normal after a few weeks exposure to normal oxygen pressure. Boycott and Oakley (21) noted a fall in the number of reticulocytes in rats which were placed in atmospheres providing 50 to 100 per cent oxygen. They interpreted this result as indicating a depression of erythrocytogenesis, but were unable to demonstrate the development of an anemia. Karsner (32) has studied the pathologic effects of atmospheres containing 80 to 96 per cent oxygen administered to rabbits for from 1 to 4 days. He used as controls both rabbits exposed to room air and rabbits exposed to low partial pressures. The experimental animals developed a fibrinous bronchopneumonia in from 24 to 48 hours. Specimens of bone marrow from 10 of these animals showed no distinct departure from the appearance in the controls. The total length of exposure to high oxygen concentrations, however, had been short.

Barach and Richards have also followed the blood counts of 2 human subjects with pulmonary tuberculosis who were kept in an oxygen tent (50 per cent) for 1 to 4 months (33). One of the patients had a red blood cell count of 5,080,000 and a hemoglobin of 90 per cent at the time oxygen therapy was begun. At the end of 5 weeks in 50 per cent oxygen, his red blood cell count was 5,010,000, hemoglobin 92 per cent. The second patient had an initial erythrocyte level of 6,340,000 cells and 121 per cent hemoglobin; these values fell to 5,400,000 red cells and 95 per cent hemoglobin during oxygen therapy. Barcroft, Hunt, and Dufton studied 26 patients with carbon monoxide poisoning who were placed in oxygen chambers (40 to 50 per cent oxygen) for 16 to 17 hours a day for 5 days (34). In those cases in which the red blood cell count was markedly higher than 5,000,000 cells, it was reduced to approximately that level during residence in oxygen; the other patients showed no

change. It will be noted that the only subjects in these 2 investigations who showed any decrease in red blood cells were those who initially had a moderate erythrocytosis, and all of them had complicating factors which tended to interfere with the oxygenation of hemoglobin. The possibility certainly exists in these instances that the moderate erythrocytosis was caused by a relative anoxemia and disappeared when the anoxemia was corrected. These observations, therefore, are not strictly comparable to the ones described in this report. Furthermore, since the concentrations of oxygen used by Barach and Barcroft were relatively low, the failure to demonstrate a clear cut depression of erythrocytogenesis is not surprising.

Patients with a chronic hemolytic anemia, like sickle cell anemia, are exceptionally suitable subjects for this type of investigation. The presence of a relatively high reticulocytosis under "basal" conditions makes it possible to detect early any depression of the rate of red blood cell formation, since the depression would be accompanied by a decrease in the reticulocytes. Furthermore, because of the hemolysis, the erythrocytes have a shorter average life span. The lowered rate of erythrocytogenesis is, consequently, more promptly reflected in the development of an anemia than would be the case under normal circumstances. If similar depression were produced in an individual whose red blood cells are not being destroyed at an abnormally rapid rate, oxygen administration would probably have to be continued for 4 or more weeks before the fall in the red cell count could be detected. This consideration is probably responsible for the failure of Barach and McAlpin (35) and others to lower the erythrocyte levels of patients with polycythemia vera by oxygen administration. Hempelmann, *et al.* (36) treated 15 patients with polycythemia vera with radioactive phosphorus. The interval from the administration of a single large dose of radioactive phosphorus to the onset of a significant decline in the red blood cells and hemoglobin levels ranged from 33 to 61 days. Hence, in patients with polycythemia vera, well over a month may elapse before a depression of erythrocytogenesis is manifested in the peripheral blood. We have also obtained entirely negative results when high concentrations of oxygen were given for 12 days to a patient with true polycythemia. It is recognized that these observations need to be extended to other hematologic dyscrasias, particularly to other types of hemolytic anemia, before it can be concluded that the changes described are not peculiar to sickle cell anemia.

In order to explain the depressant effect on bone marrow of the high partial pressures of oxygen in alveolar air and in arterial blood, one must assume that these high tensions are transferred, at least in part, to the marrow itself. Since oxygen saturation of both arterial and venous blood was shown to be increased, the bone marrow and all the other tissues of the body must have been exposed to oxygen at a partial pressure somewhat greater than the normal. That this increase in the oxygen tension of the tissues is considerably greater than appears from the increase in the oxygen content of the arterial blood is indicated by the researches of Campbell (37). Campbell found that when rabbits and cats breathed 90.77 per cent oxygen, the oxygen tension of the abdominal cavity rose from a normal level of about 30 mm. Hg to more than 50 mm. Hg. This relatively large increase develops for 2 reasons: (*1*) the oxygen tension of tissues is proportional to the oxygen tension of the blood; and (*2*) the oxygen tension of blood rises rapidly once hemoglobin has become saturated and extra oxygen is physically dissolved in the plasma. When pure oxygen is breathed and hemoglobin has become changed to oxyhemoglobin, more oxygen physically dissolves in plasma until the oxygen tension in the plasma approximates that in the alveoli (Henry's law for the solution of gases in liquids). Consequently, since the oxygen tension in alveoli increases during periods of pure oxygen administration from a normal of about 100 mm. Hg to over 500 mm. Hg, the small increase in oxygen content of arterial blood over so-called oxygen capacity (physically dissolved oxygen) produces a severalfold increase in oxygen tension of arterial blood. It is because of this relatively high oxygen tension of blood as it enters the arterial end of the capillaries that the normal oxygen tension of tissues is nearly doubled (37). Similar conclusions were reached by Sibree (38). The assumption may be made, therefore, that the oxygen

tension in the bone marrow was probably raised significantly when the 4 patients used in the present study were given oxygen through a B.L.B. mask. With the increase in oxygen tension, utilization of oxygen may actually be decreased because of toxic effects rather than increased (39).

Reference should be made to the claim of several investigators that plasma volume is slightly increased during periods of intensive oxygen therapy (28, 40). Hydremia would cause a slight (relative) lowering of the red blood cell count and hemoglobin values. Simple dilution of the formed elements, however, could not account for a major portion of the changes observed in the patients here reported; the fall in erythrocytes was occasionally as great as 50 per cent. Furthermore, the alterations in total number of red cells were correlated so closely with other evidences of changes in bone marrow activity that the latter certainly must have been the principal controlling factor.

X. *Summary and conclusions*

During 6 periods of 8 to 20 days each, 4 patients with sickle cell anemia breathed 70 to 100 per cent oxygen administered through a Boothby-Lovelace-Bulbulian mask. The following effects were observed:

(*1*) A decrease in the degree of intravascular sickling of red blood cells occurred.

(*2*) No consistent detectable change in the rate of hemolysis occurred during the period of oxygen administration.

(*3*) *Erythrocytogenesis was depressed by the oxygen therapy.* This depression was reflected in a decrease of both reticulocytes and red blood cells. After oxygen inhalation was discontinued, a striking reticulocytosis developed and the level of erythrocytes returned to the pre-oxygen level. It is suggested that the increased oxygen tension produced in bone marrow by the oxygen administration was responsible for the depression of erythrocytogenesis, and that this effect is the physiological antithesis of the stimulation of red cell formation produced by low oxygen tensions.

(*4*) Leukocytes and platelets showed no significant numerical or qualitative change. However, after oxygen administration was discontinued, an increase in the number of circulating leukocytes, presumably due to an outpouring from the bone marrow, was regularly observed.

(*5*) Only minor toxic manifestations developed during the periods of oxygen therapy.

Case reports

G. C.

A colored female, 18 years old, had complained for years of recurrent pains in the legs and arms, pain and swelling of the joints, abdominal pain associated at times with nausea and vomiting, and fever. Moderate jaundice had been noticed on 2 occasions. Because of the recurrent joint pain and the presence of a loud apical systolic murmur, several physicians had told her she had rheumatic heart disease. The diagnosis of sickle cell anemia was first made when she was 9 years old. Shortly thereafter, a splenectomy was performed without apparent effect on the symptoms or the degree of anemia. When the present studies on this patient were begun, she had some pain in both thighs but no other symptoms; 2 weeks previously her left ankle had been extremely painful and swollen but this had subsided within 3 or 4 days.

Physical examination revealed a pale young negress with a small ulcer on her left ankle as well as the scar of an old healed ulceration. The sclerae were slightly icteric. The teeth were carious and the breath fetid. The heart was enlarged to percussion, rhythm was regular, and there was a soft blowing systolic murmur heard best at the apex but also audible at the base. The abdomen was negative except for left and right paramedian scars. The left wrist was slightly swollen and painful on motion.

Laboratory data: Red blood cell count was 2,330,000; hemoglobin 7.6 grams, leukocyte count 16,500; reticulocytes 18 per cent. In formalin-fixed venous blood, 53 per cent of the erythrocytes were sickled. The icterus index was 10.

A lateral x-ray of the skull showed a granular appearance of the bones. An electrocardiogram was normal.

Symptoms during oxygen inhalation: 12 hours after oxygen was started, the pain in her left thigh disappeared and did not recur during the period of oxygen inhalation. On the eighth and last day of oxygen inhalation, she developed a sore throat. The following day her temperature rose to 102°F. That evening her left thigh began to ache again. The sore throat and fever lasted only 3 days but pains grew progressively worse and 4 days after oxygen was stopped, she had very severe pains in both legs and in her back. These gradually subsided over a period of 4 to 5 days.

L. McC.

A colored female, 25 years of age, had been admitted to Barnes Hospital 7 times between March, 1939, and March, 1942. All of the oxygen studies were done during her last admission which was for a period of 9 weeks. At various times, she had had abdominal cramps, pain in the extremities, pain under the sternum, and fever. In 1939,

delivery of a premature infant was followed by post-partum bleeding. This finally ceased 3 months later following currettage. During the latter months of her pregnancy, an incorrect diagnosis of rheumatic heart disease with mitral stenosis was made. The patient had had pain in the back many times, and, during one of her hospital admissions, she remained in the opisthotonic position screaming with agony for several days. It was necessary to administer avertin by rectum to relieve the muscle spasm and pain. When admitted to Barnes Hospital in March, 1942, for oxygen studies, she had pain in the back and legs, but by the time oxygen administration was actually started she had been entirely asymptomatic for 4 days. Furthermore, she had no symptoms referable to her sickle cell anemia in the interval between the first and second periods of oxygen administration.

Physical examination: The patient was a thin but well developed young negro girl. The skin was clear, the mucous membranes pale. Fundi normal. The heart was moderately enlarged and a blowing systolic murmur was heard over the whole precordium, loudest at the apex. The lungs were clear. Spleen was not palpable.

Laboratory data: Red blood cell count was 3,330,000, hemoglobin 9.3 grams, white blood cell count 19,500, reticulocyte count 21.6 per cent, platelets 980,000. The leukocyte differential was normal. Formalin-fixed venous blood contained 47.1 per cent sickled erythrocytes. The urine was normal, and the Kahn reaction was negative. The icterus index was 6.

Lateral x-ray films of the skull showed a granular appearance of the frontal and parietal bones. The electrocardiogram was normal.

Symptoms during oxygen inhalation: 3 days after oxygen was started, a peculiar numbness and stiffness of both hands were noted. Twenty-four hours later the patient complained of tiny sharp shooting pains in her abdomen, shoulders, and extremities. These differed from any pains she had had in the past in that they lasted only a fraction of a second. The morning of the fifth day of oxygen inhalation she had sharp shooting pains across the whole anterior aspect of her chest. That afternoon she had a headache and the next day this was very intense. Her hands continued to feel numb and her right shoulder became similarly affected. She stated she had never experienced any comparable sensation in the past. All of these symptoms except the numbness of the hands disappeared, and during the last 3 days of oxygen, she felt well except for a feeling of exhaustion. Her appetite fell off progressively and during the 12 days she wore the B.L.B. mask she lost 8 pounds in weight. During the second oxygen period, this one also lasting 12 days, she developed no symptoms except stuffiness of the nose on the second day and headache on the eighth day. In neither case was the discontinuance of oxygen associated with any notable symptoms.

T. S.

A colored female, 21 years of age, at the age of 9, had had fever with pain and swelling of her ankles, knees, and elbows. These symptoms lasted 3 weeks and the diagnosis of acute rheumatic fever was made. At the age of 16, she developed abdominal pain and pains in her arms, legs, and back. The joints were not swollen. At about the same time, ulcers appeared on both lower legs. The pains gradually disappeared but have recurred at intervals since then. As soon as the leg ulcers healed, new ones appeared. She has had frequent sore throats for the last 4 years.

Physical examination: The patient was pale, thin, and underdeveloped. On ophthalmoscopic examination, the vessels of the fundi were dilated and tortuous. The tonsils were large and chronically inflamed. The heart was not enlarged. A blowing systolic murmur was heard over the whole precordium, loudest at the apex. There were no signs of valvular disease. The spleen was not palpable. Small ulcers were present on the medial aspect of both legs just above the internal malleoli.

Laboratory data: Erythrocyte count was 1,840,000, hemoglobin 6.4 grams, white blood cell count 10,150, reticulocyte percentage 32.6, platelets 960,000, leukocyte differential normal. Urine was normal. Kahn negative. In formalin-fixed venous blood, 51 per cent of the erythrocytes were sickled.

Lateral and antero-posterior x-rays of the skull were normal as were films of all the long bones of all 4 extremities. An electrocardiogram was normal except for an inverted T-wave in lead CF-4.

Symptoms during oxygen inhalation: No symptoms other than weakness were present when oxygen inhalation was begun. Within 48 hours, anorexia developed. By the third day, the patient was nauseated. Nausea became worse until the twelfth day when she began to vomit. She complained of a very bad taste in her mouth.

On the third day, dizziness and light-headedness appeared. Three days later, she had a slight headache. This increased until by the ninth day her headache was intense. She began to complain of numbness and tingling of the hands and feet on the fifth day. By the ninth day, the hands and feet felt stiff and her calf muscles were sore.

She began to cough on the tenth day and the following day brought up thick mucoid sputum. The mucous membranes of the nose and throat appeared very pale; the lungs were clear to both physical and x-ray examination.

By the morning of the thirteenth day, oxygen administration had to be discontinued because of the intensity of her headache and the constant vomiting. Her cough had improved somewhat but she was still clearing much phlegm from the back of her throat.

After oxygen inhalation was stopped, the patient's nausea and anorexia began to subside within 3 days and by the fifth day were no longer present. The headache, however, persisted for a full week. She had several nosebleeds and a transient sore throat without visible inflammation during the first few post-oxygen days.

B. W.

This colored female was 20 years old. When first seen at the Barnes Hospital in September, 1941, this patient gave a history of 10 previous admissions to other hospitals. Her complaints at various times had been abdominal pain with vomiting, pain in the extremities, swelling of the

legs, pain and swelling of various joints, and fever. On all her hospital admissions, a systolic murmur had been heard at the apex and, on several occasions, the incorrect diagnosis of rheumatic fever and rheumatic heart disease had been made. When oxygen administration was started the first time, the patient had no symptoms, but one week previously she had had an attack of pain without swelling of the right knee. When she was again hospitalized, 7 months later, for a repetition of the oxygen studies, she had just recovered from an attack of severe pain in both legs and mild pain in the shoulders.

Physical examination: Patient was slight of build, rather thin, and pale. The skin was clear. Fundi appeared normal. There was slight icterus of the sclerae. The heart was not enlarged. A soft blowing systolic murmur was heard over the whole precordium. The lungs were clear. Spleen was not palpable. There was no swelling or deformity of any of the joints. The left leg was larger than the right; the left calf measured 37 cm. in circumference while the right calf measured 31 cm. The 2 legs were equal in length.

It was thought that the enlargement of the left lower extremity was the result of a thrombosis of the left femoral vein.

Laboratory data: Erythrocyte count was 2,210,000, hemoglobin 9.6 grams, white blood cell count 13,200, reticulocytes 20.4 per cent, platelets 670,000. The differential count was essentially normal. In formalin-fixed venous blood, 37.3 per cent of the erythrocytes were sickled. The urine contained a trace of albumin. The Kahn reaction was negative. The icterus index was 30.

A lateral x-ray of the skull showed a poorly defined rounded area of destruction in one of the parietal bones. An electrocardiogram was entirely normal.

Symptoms during oxygen inhalation: No pains of any sort were present during either period of observation. She was given oxygen the first time for 8 days, the second time for 20 days. On the fifth day of the first oxygen period, she developed stuffiness of the nose, post nasal discharge, and a severe headache. The next day she was hoarse and complained of a burning sensation in the nose and throat. Examination revealed only swelling and edema of the mucous membranes without other evidence of inflammation. These symptoms lasted 3 days. Her appetite was poor. The day after oxygen was stopped she again had a headache, and the following day she was bothered by slight epigastric discomfort with nausea and vomiting, but this lasted only one day. She had a mild nosebleed. Four days after oxygen was stopped she felt fine and remained asymptomatic until her discharge several weeks later. Beginning about the eighth day of the second oxygen period, the patient again had stuffiness of the nose, impaired hearing, smarting of the eyes with lacrimation, and hoarseness. There were swelling and edema of the mucous membranes of the nose, throat, and larynx without redness. These symptoms cleared up in about 5 to 6 days in spite of the continuance of oxygen administration. The day after oxygen was stopped she had a headache, profound weakness, and complete anorexia. She vomited repeatedly the following day. Two days later she felt perfectly well and remained asymptomatic thereafter.

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HEBERDEN'S NODES: THE MECHANISM OF INHERITANCE IN HYPERTROPHIC ARTHRITIS OF THE FINGERS[1]

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In 2 previous papers, Heberden's nodes were described, the incidence of their occurrence in the general population was presented, and evidence was offered leading to the conclusion that this condition is hereditary. It is our purpose at the present time to offer additional data and to subject the material to modern methods of statistical and genetic analysis in order to discover the probable mechanism of inheritance of this form of joint disease.

Heberden's nodes are enlargements of the terminal interphalangeal joints of the fingers, due to hypertrophic arthritis (1). A survey of a cross-section of the general population showed that the condition sometimes occurs as a result of direct trauma to the fingers. Enquiry served to distinguish such nodes from another type arising spontaneously, the so-called idiopathic Heberden's nodes which are the subject of the subsequent discussion. The incidence of idiopathic Heberden's nodes was found to be higher in white people than in Negroes, it was higher in women than it was in men, and it increased markedly with age.

The second study (2) based upon 68 families demonstrated that the mothers of affected women showed the condition twice and the sisters 3 times as commonly as the women in the general population. In a control series, the sisters of unaffected women were involved as frequently as the population in general. Three combinations of familial involvement which were discovered in this series could not be expected to occur as a result of chance alone more frequently than once in 190 families for the first, once in 4,500,000 for the second, and once in 10,000,000 families for the third. An hereditary factor influencing the production of the condition in women seemed to be established.

The present study is based upon the pedigrees of 74 affected persons. The affected index cases and their siblings include 127 men and 215 women. The difference in the number of men and women in the group is due to the fact that 72 of the 74 index cases were women. When the index cases are subtracted from the total group, there remain 125 men and 143 women, a fair approximation to the proportions of men and women in the general population of this age distribution.

Examination of the pedigrees shows at once that the incidence in women is high compared to that in men. For this reason, each sex will be considered separately in the genetic analysis. Considering first the women, it is found that 108 of the 215 women, or one-half, are affected, the 1:1 ratio suggesting immediately that the character depends upon a single autosomal dominant gene. When this is the case, one parent is invariably expected to be affected. Actually, mothers are recorded affected in 25 of the 74 families. In 2 families, the maternal grandmother was involved but the mothers, dying before the ages of 35 years, were spared. Only 1 father was recorded affected. This family added nothing to the genetic analysis because the mother was also affected and only 1 daughter, the index case, resulted from the match. In 4 families without the mother affected, there is reason to believe that the character was transmitted through the unaffected father. In 2 instances, the paternal grandmother and in 2 other instances, a paternal aunt had Heberden's nodes. In 11 families, including the 2 mentioned above, the mothers died before the age of 50 so might have been genetically affected without demonstrating the character. In 4 families, women were affected in 3 successive generations. Of the

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74 families, some antecedent involvement was discovered in 42 instances but it was lacking in 32 others.

Although Heberden's nodes may seem to be dominant in women, they certainly are not so in men. Of the 127 males of this series, only 4 are recorded affected. They may be recessive. Genetic examples are recognized of factors dominant in one sex and recessive in the other. These include color variations in cattle, the development of horns in sheep, and hereditary baldness in humans. Baldness is dominant in men and recessive in women (3). With the heterozygous constitution (Aa), men develop the trait and transmit it to one-half of their offspring. Women with this constitution do not exhibit the condition but transmit it to one-half of their children. Women become bald only when they are homozygous (AA). With Heberden's nodes, the simplest modification is to assume that the condition is recessive in men. With matings of the Aa x aa type, one-half of all children will be heterozygous (Aa). The women of this constitution will develop the condition while the men will not. The men who are affected would be homozygous (AA). According to this assumption, an equal number of men and of women are of the heterozygous (Aa) constitution and such men may have been the fathers in those families where no antecedent involvement was known. Furthermore, for a man to be affected, both parents must transmit the character to him but only the mother is likely to show it.

The above hypothesis was tested by gene frequency analysis. For the subsequent discussion, *D* will be used to represent the dominant gene for Heberden's nodes and *d* the recessive normal allele. In the former study, the highest incidence in white women over 70 years was found to be about 30 per cent. If this is accepted as complete penetrance, we may assume that 70 per cent of women are homozygous recessives and are *dd* in constitution, or completely normal so far as Heberden's nodes are concerned. The proportion of the 30 per cent who are *Dd* and *dd* in constitution may be readily calculated from the well-known formula for gene frequency analysis in a population in genetic equilibrium mating at random. According to the formula, homozygous dominants *DD*, heterozygous *Dd*, and homozygous recessives *dd* are present in the population in the relative numbers q^2, 2pq, and p^2, respectively, where p plus q equals 1. Since p^2 is 0.70, the value of p is 0.837 from which q is readily known from the relation p plus q equals 1. The values of 2pq and q^2 are easily calculated. The result shows that individuals with the constitutions *DD*, *Dd*, and *dd* are present in the population with the relative proportions of 0.027, 0.272, and 0.70, respectively. According to this hypothesis, males of the constitution *Dd* are phenotypically normal so that only 2.7 per cent of males have a genetic constitution which would cause the trait to develop.

In the original survey of the population in general, a rapid rise in incidence in females was found from 2.6 per cent at the sixth decade to an approach to an upper asymptote of nearly 30 per cent in the ninth decade. In males, the incidence rose from 3.6 per cent in the sixth decade to 8.4 per cent in the eighth decade with no approach to an asymptote. In a small sample in the ninth decade, an incidence of 18.2 per cent was found. Because the incidence varied so markedly from one sex to the other and the incidence found in the original survey was so different from the theoretical expected from the gene frequency analysis, a second survey was undertaken, confined to white men and women 70 years or more of age. Every patient of this description admitted to City Hospital was examined, as well as the inmates of 4 homes for the aged. In the course of 3 months, 151 men and 184 women were observed and the results tabulated in Table I. The incidence in women was very close to the original estimate, substantiating the previous observations and strengthening the

TABLE I

The incidence of Heberden's nodes—Second series
White men and women, 70 years of age or older

Age	Men			Women		
	Subjects examined	Number affected	Percentage affected	Subjects examined	Number affected	Percentage affected
70's	124	4	3.2	117	28	23.9
80's	24	1	4.2	58	21	36.2
90's	3	0		9	3	33.3
Total	151	5	3.3 ±1.45	184	52	28.3 ± 3.3

contention of an ultimate incidence of 30 per cent after complete penetrance. The observation of men was at marked variance from the original findings, only about 3 per cent affected being found instead of 8 to 18 per cent. Increased experience with the condition over a period of 6 years served to eliminate more accurately the cases of traumatic nodes which had previously been included.

Since the proportions of the various constitutions for Heberden's nodes are known, the types of matings which can be expected to occur at random can be predicted. They are given in Table II. The top figure gives the proportion of each type of mating expected and the second figure gives the actual number of each type of mating expected in this group of 74 families. Since each pedigree was discovered through an affected child and never from a parent, no mating of type dd x dd is included. The frequencies of

TABLE II

Types of mating for Heberden's nodes

First number in each cell is the proportion in the population. Second figure is actual number expected in this series of 74 families.

		Constitution of the mothers		
		DD $q^2=0.027$	Dd $2pq=0.272$	dd $p^2=0.70$
Constitution of the fathers	DD $q^2=0.027$	0.00073 0.1	0.0073 1.1	0.0189 2.8
	Dd $2pq=0.272$	0.0073 1.1	0.074 10.8	0.1904 28.
	dd $p^2=0.70$	0.0189 2.8	0.1904 28.	0.490 None

the 8 different types of pedigrees with the genetic constitutions of the parents are shown in Table III. The matings are sorted into 4 groups depending on whether both parents, the mother alone, the father alone, or neither parent has the constitution for the development of Heberden's nodes according to the hypothesis being tested.

Although it is not possible to recognize the different genotypes with certainty in all families, an attempt at rough classification is justified. According to Table III, 44 families with inheritance from the mother are expected. Of these, 29 are identified with a fair degree of certainty. These include 25 families with mother affected,

TABLE III

Frequency of matings of phenotypes among 74 families with expected proportion of affected children

Dominant in women, recessive in men

Phenotype of parents	Genotype of parents		No. of families of this series	Proportion of children affected	
	Mother	Father		Sons	Daughters
Both parents affected	DD Dd	DD DD	0.1 1.1	All One-half	All All
Mother only affected	DD DD Dd Dd	Dd dd Dd dd	1.1 2.8 10.8 28.0	One-half None One-fourth None	All All Three-fourths One-half
Father only affected	dd	DD	2.8	None	All
Neither parent affected	dd	Dd	28.0	None	One-half

2 families with maternal grandmother affected, and 2 additional families with affected sons. Assuming random mating, 12 heterozygous fathers are expected in this group of 44 families, leading to double inheritance and a 3:1 ratio of expected affected. Of the 29 families identified, therefore, we expect an appreciably greater frequency than the straight 1:1 ratio.

According to the table of matings, 28 families are expected with no parent showing the trait. Actually, 35 families were found without suspected inheritance from the mother. It is concluded that inheritance is through the father and the ratio of 1:1 affected is expected. Ten additional families have been omitted from the above classifications because the mother died so young that her constitution could not be recognized.

The above ratios are theoretical and cannot be realized in material such as is dealt with here. Corrections can be made for small family size and for lack of penetrance. The desirability of the first is obvious. Those families, of proper matings to transmit the character, which have only normal children, must be counted to attain expected proportions of affected and unaffected children. The corrections are made according to the method of Hogben (4).

In Table IV are 29 families with known maternal inheritance. Of 95 daughters, 53 are observed affected compared to 54.5 ± 4.16 ex-

TABLE IV

Comparison of affected daughters with theoretical expectation on basis of simple autosomal dominant (1 : 1 ratio)

Corrected for family size, in families with mother or maternal ancestor known to be affected

No. of daughters	No. of families	Total daughters	Observed affected	Corrective factor	Expected 1:1 ratio	σ^2*
1	5	5	5	1.0	5.000	0.000
2	8	16	10	1.333	10.664	1.778
3	3	9	5	1.714	5.142	1.469
4	5	20	10	2.133	10.665	3.911
5	5	25	8	2.581	12.905	5.410
6	2	12	8	3.048	6.096	2.758
8	1	8	7	4.016	4.016	1.945
Totals	29	95	53		54.488	17.271 $\sigma \pm 4.16$

* The term σ^2, also called variance, is the square of the standard deviation. Differences between observed and expected results greater than 3 times the standard deviation cannot be expected to occur by chance alone more often than about 3 times in 1000. The figures for variance in Tables IV and V are taken from Hogben.

pected affected. In Table V are 35 families without maternal inheritance. Of 96 daughters, 46 were found affected compared to 57.6 ± 3.9 expected. The difference is 11.6 or 3 times the standard deviation. It is seen that a higher proportion of affected is found in the group with suspected double inheritance than in that with single inheritance.

Further correction must be made for lack of penetrance, and is attempted as follows. The onset of the condition is insidious and an exact date of first appearance is rarely remembered by the patient. However, in 95 instances in women, an approximate age of onset seemed reasonably reliable. The age of onset ranged from 30 to 65 years. The mean age of onset was 48.5 years; the median age was 49.2 years. Three-fourths of these women were affected by the age of 54.3 years. Applying this information, Table VI was devised. It will be seen that in the first group, 66.3 are finally expected affected compared to 53 actually found. In the second group, 53.6 are finally expected affected compared to 46 actually found. In the first group, after correction for incomplete penetrance, 70 per cent of the daughters are finally expected affected compared to 56 per cent in the second group. Correction for incomplete penetrance can be computed in another manner leading to essentially the same results. In the group with known affected maternal ancestors, there are 46 women above the age of 55 years when penetrance may be considered complete. Of these, 33, or 72 per cent, are affected. If this proportion of affected is applied to the entire group, the result is 68.4 as compared to 66.3 previously found. In the group without inheritance from the mother, 63 women are 55 years or older and of these, 35 or 56 per cent are affected. Fifty-six per cent of 96 is 53.7 compared to 53.6 previously obtained. Thus it is seen that corrections for incomplete penetrance, made in two different manners, produce the same result.

After the above corrections, it is seen that in 29 families with known maternal inheritance, and suspected paternal inheritance in about one-third of them, 70 per cent of the daughters are finally expected affected. In 35 families without suspected maternal inheritance and with paternal inheritance only, 53.6 per cent of the daughters are finally expected affected. The latter is very close to the theoretical 1:1 ratio while the other

TABLE V

Comparison of affected daughters with theoretical expectancy on basis of simple dominant (1 : 1 ratio), and since neither parent shows the trait, also on basis of 3 : 1 ratio

Corrected for family size

No. of daughters	No. of families	Total daughters	Affected daughters	1:1 ratio			3:1 ratio		
				Factor	Expected	σ^2	Factor	Expected	σ^2
1	10	10	10	1.000	10.	0.000	1.	10.	
2	3	6	3	1.333	4.000	0.666	1.143	3.429	0.367
3	11	33	13	1.714	18.854	5.388	1.297	14.067	2.893
4	8	32	14	2.133	17.064	6.157	1.463	11.704	3.360
5	3	15	6	2.581	7.743	3.246	1.672	5.017	1.776
	35	96	46		57.661	15.457 $\sigma = \pm 3.9$		44.217	8.396 $\sigma = \pm 2.9$

TABLE VI

Correction for incomplete penetrance

Age group, years	Degree of penetrance	29 families with known affected maternal ancestor			35 families without known affected maternal ancestor		
		Total daughters	Affected daughters	Finally expected affected	Total daughters	Affected daughters	Finally expected affected
30 to 48	One-half	24	10	20	24	6	12
49 to 54	Three-quarters	25	10	13.3	9	5	6.6
55+	Complete	46	33	33	63	35	35
		95	53	66.3 70 per cent	96	46	53.6 56 per cent

is over 2:1. In 4 families with known double inheritance and with affected sons, we expect a 3:1 ratio of affected daughters. Of 11 daughters in these families, 8 were known to have been affected. Even without correction this is very close to the theoretical expectancy of 3:1.

The second group of pedigrees mentioned above may be used for a further test of the hypothesis that the trait is dominant in females but recessive in males. In this group of 35 pedigrees, neither parent shows the trait although some of the children do and so conform to the rule of thumb for the identification of a simple autosomal recessive characteristic. By the hypothesis under scrutiny, these matings are of type 8 dd x Dd and so the father, although phenotypically normal, is heterozygous in genetic constitution. The ratio of expected affected among the daughters is 1:1 in contrast to 3:1 if the trait is recessive. The numerical test of these opposed assumptions is made in Table V. It may be seen that, after correction for incomplete penetrance, 53 are finally expected affected. On the basis of dominance, 57.7 ± 3.9 and on the basis of a recessive, 44.2 ± 2.8 are expected. These data more nearly conform to and support the theory that the trait is dominant rather than recessive.

The hypothesis that Heberden's nodes are recessive was further tested. For this purpose, let N denote the normal dominant gene and n its recessive allele. The approximately 30 per cent of women who show the trait when penetrance is complete are nn in constitution. Applying the equation for gene frequency analysis, the relative numbers in the population of homozygous normals, NN, heterozygotes, Nn, and homozygous recessives, nn, are 0.204, 0.495, and 0.30, respectively. Assuming random mating, the frequencies of those types of matings capable of having children susceptible to Heberden's nodes among the 74 pedigrees are given in Table VII. It will be noted that 28 pedigrees are expected with the father affected compared to 4 expected under the other theory and one actually found. Moreover, 10 pedigrees are expected with both parents and all the offspring affected. Actually but one was found. We expect to find 17 families with the mother affected but 29 are found. We expect only 29 families with no parent affected but 35 are found. On the hypothesis of a recessive, the sex difference must be regarded as arbitrary. The observed data lend no support to the theory that Heberden's nodes are recessive.

CONCLUSIONS

Heberden's nodes show certain peculiarities from the genetic standpoint. These include (*1*) a great preponderance among women, about 30 per cent compared to 3 per cent in men, or a ratio of 10:1 in the general population; (*2*) multiple involvement among female sibships without broth-

TABLE VII

Frequency of matings of phenotypes among 74 families with expected proportions of affected children

Simple recessive

Phenotype of parents	Genotype of parents		No. of families of this series	Proportion of children affected	
	Mother	Father		Sons	Daughters
Both parents affected	nn	nn	10.5	All	All
Mother only affected	nn	Nn	17.4	One-half	One-half
Father only affected	Nn	nn	17.4	One-half	One-half
Neither parent affected	Nn	Nn	28.7	One-fourth	One-fourth

ers being affected; (*3*) transmission through females in a manner to suggest maternal unilateral inheritance; (*4*) a greater frequency in families with mothers involved; (*5*) fathers rarely affected; (*6*) approximation to 1:1 ratio among the daughters in families where neither parent is affected; (*7*) a high incidence in sisters of affected men. In spite of the apparent lack of agreement with mendelian ratios, the analysis shows, on the basis of numerical tests, that the data support the hypothesis that the genetic mechanism involves a single autosomal gene, sex influenced, dominant in females and recessive in males.

This hypothesis accounts satisfactorily for all the genetic peculiarities of Heberden's nodes as exhibited by the 74 pedigrees. The unusual feature of the simple mechanism postulated is the effect of sex on dominance. Dominance is a phenotypic property which has long been recognized to be readily modified by the genetic background and also by environmental factors. It is consistent with current opinion of physiological genetics to suppose that the effective or threshold concentration of some substance necessary for a reaction to occur, or of a substance which prevents a reaction from occurring, can be altered in such a way as to allow for an autosomal gene to be dominant in one sex and recessive in the other.

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THE NUTRITIVE VALUE OF HUMAN PLASMA FOR THE RAT

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Extensive studies by Madden and Whipple (1) on the value of various proteins in the regeneration of plasma proteins in dogs have demonstrated a marked superiority of plasma proteins over all other protein sources tested. Similar data by different technics have been obtained by Weech (2) and by Melnick, Cowgill, and Burack (3), although the latter group concluded that the differences between serum, casein, and lactalbumin were not significant. It thus appears that plasma proteins may be considered to be of high biological value as measured by these technics. In measurements of the hematopoietic values of certain proteins for rats, Orten and Orten (4) found that beef serum was much less effective than lactalbumin, and when fed at a level which supplied 18 per cent of protein in the diet, supported only very poor growth. This is in marked contrast to the above results and might be unexpected if one considers the plasma proteins as a labile source of protein for other body functions.

Although plasma proteins are not used as a source of human food, their nutritive value may be of considerable importance. Patients are often maintained for some time with plasma as the chief if not the sole source of protein. Protein appears to be of primary importance in many of these patients, and the huge losses of nitrogen after various types of injury have recently been stressed (5). Recent data also emphasize the very rapid response of the body to amino acid deficiencies, negative nitrogen balances being obtained within a few hours (6, 7). The implication of these combined facts is that if patients are unable to take adequate diets, the intravenous nutrients, especially protein, should be as adequate as possible.

The studies reported in this paper were undertaken to determine the nutritive value of human plasma proteins for the young rat and have shown that isoleucine is the chief deficiency of these proteins for this animal.

EXPERIMENTAL

Young male weanling rats, weighing 30 to 35 grams, were used in all of these studies. They were placed immediately upon a purified ration containing the appropriate protein supplement, corn oil 4 per cent, salt mixture 4.7 per cent,[1] all of the crystalline vitamins[2] in excess of requirements (8) and sucrose to make 100 per cent. Haliver oil was fed by dropper twice weekly. For comparative purposes, various levels of casein were fed as the sole source of protein. Dried plasma[3] was fed to supply 18 per cent of protein and when amino acid or protein supplements were fed with the plasma, it was correspondingly decreased so that the total protein ($N \times 6.25$) was 18 per cent of the ration.

Two experiments were run to determine the supplementary effect of casein, zein, and gelatin on plasma proteins. As shown in Table I, very

TABLE I

The value of various proteins in supplementing human plasma in rat-growth experiments

Kind and amount of protein	Number of rats	Duration	Gain per day
		days	*grams*
12 per cent casein	5	33	2.21
15 per cent casein	5	33	2.53
18 per cent casein	8	16 and 33*	3.33
18 per cent plasma	8	16 and 33*	0.92
3 per cent casein + 15 per cent plasma	8	16 and 33*	1.39
6 per cent casein + 12 per cent plasma	8	16 and 33*	1.87
9 per cent casein + 9 per cent plasma	3	16	2.0
3 per cent zein + 15 per cent plasma	3	16	1.25
6 per cent zein + 12 per cent plasma	3	16	1.41
9 per cent zein + 9 per cent plasma	3	16	1.67
3 per cent gelatin + 15 per cent plasma	3	16	1.08
6 per cent gelatin + 12 per cent plasma	3	16	1.12

* Two experiments of 33 and 16 days' duration gave essentially the same result.

[1] Salts IV (J. Biol. Chem., 1941, 138, 459) 4 grams and K_2HPO_4 0.7 grams.

[2] We are indebted to Merck and Co. for furnishing the crystalline vitamins used.

[3] We are indebted to Mr. E. B. Carter of Abbott Laboratories for securing dried plasma for these investigations. The plasma so obtained was contaminated and not suitable for human use. It was secured from the Army Medical Supply Office.

poor growth was obtained with plasma alone. Relatively high levels of casein are required to improve the rate of growth, and gelatin and zein were much less effective than casein. It thus appeared that none of these proteins was a rich source of the amino acids which are low in plasma.

Studies on the supplementary action of amino acids are shown in Table II. In these studies, all of the animals were first fed the ration containing only plasma as a source of protein for 3 to 5 days. They were then divided into comparable groups and fed the experimental ration. The growth obtained with 18 per cent casein is usually about 3 grams per day. In Experiments I and II, the growth obtained with plasma supplemented with a mixture of amino acids was good and approached that obtained with casein. Further studies in Experiments III and IV show that the deficiency in human plasma proteins is due chiefly to a lack of sufficient isoleucine. Apparently, 0.5 per cent additional dl isoleucine is about as effective as is 1 per cent. Since only small numbers of animals have been used (because of the expense of amino acid supplementation), the differences between the various groups receiving isoleucine and those receiving isoleucine plus other amino acids are of doubtful significance, although the further addition of arginine has always given somewhat better growth than isoleucine alone.

DISCUSSION

Analyses for 12 amino acids in serum proteins have been presented by Block and Bolling (9). A calculation of the amount of these acids which would be supplied in the ration when 18 per cent of plasma protein is used shows that all of the essential amino acids would be present in amounts equal to those reported as minimal for the growing rat (10). A similar comparison of the data given for casein indicates that plasma proteins are as high or higher than casein in all of the essential amino acids with the one exception of isoleucine. The isoleucine content of plasma proteins is given as 3.2 per cent whereas casein is reported to contain 5.1 per cent. Plasma pro-

TABLE II

The effect of amino acid supplements on the nutritive value of plasma in rat-growth experiments

Protein supplement*	No. of rats	Depletion period	Experimental period	Gain per day	No. of rats	Depletion period	Experimental period	Gain per day
		days	*days*	*grams*		*days*	*days*	*grams*
	EXPERIMENT I				EXPERIMENT II			
Plasma	3	5	10	1.12	3	4	11	1.35
Casein	3	5	10	3.55	3	4	11	2.97
Plasma + 0.9 per cent l(−) leucine, 1.0 per cent dl isoleucine, 1.4 per cent dl valine, 0.2 per cent l(−) tryptophane, 0.2 per cent l(+) arginine·HCl, and 0.6 per cent dl methionine	3	5	10	3.27	3	4	11	2.45
	EXPERIMENT III				EXPERIMENT IV			
Plasma	3	3	10	0.83	3	3	33	1.49
Plasma + 1 per cent dl isoleucine	3	3	10	2.13	3	3	33	2.72
Plasma + 0.5 per cent dl isoleucine					3	3	31	2.45
Plasma + 1.0 per cent dl isoleucine + 0.2 per cent l(+) arginine	3	3	10	2.8	3	3	33	2.96
Plasma + 0.2 per cent l(+) arginine	3	3	10	0.57				
Plasma + 1.0 per cent dl isoleucine + 0.9 per cent l(−) leucine	3	3	10	1.93				
Plasma + 1.0 per cent dl isoleucine + 1.4 per cent dl valine	3	3	10	2.40				
Plasma + 1.0 per cent dl isoleucine + 0.9 per cent l(−) leucine + 1.4 per cent dl valine	3	3	10	2.10				
Casein					3	3	31	2.86
Casein (12 per cent)					3	3	33	2.01

* All rations adjusted to contain 18 per cent protein (total N × 6.25).

teins should supply, therefore, approximately 0.57 per cent of isoleucine in a ration if fed at an 18 per cent level. Unpublished data from this laboratory obtained by microbiological assays (11) indicate an isoleucine content between 2.4 and 2.8 per cent for human plasma proteins. On the basis of these figures, an 18 per cent plasma protein ration would contain from 0.43 to 0.5 per cent isoleucine.

As far as we are aware, plasma proteins are unique among those so far investigated in that they are primarily low in isoleucine. It will be of interest to determine whether this is true of the plasma proteins from various species. According to recent evidence (12) isoleucine is also an essential amino acid for the adult human, but the quantitative requirement of the adult of no species is yet known. Thus the significance of these findings in relation to the problem of intravenous nutrition must await further investigation.

Accepting an average figure of 2.6 as the percentage of isoleucine in plasma proteins, and that only the one isomer is active (13), the isoleucine requirement of the rat would probably be near 0.7 per cent, since the addition of 0.5 per cent of dl isoleucine to the plasma proteins appears to give a near maximum response.

CONCLUSION

Human plasma proteins are inadequate as the sole source of protein for the growing rat. A low content of isoleucine is the primary amino acid deficiency. A supplement of 0.5 per cent dl isoleucine gives a near maximum response.

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THE ELECTROPHORETIC PATTERNS OF NORMAL PLASMA [1]

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Normal plasma values as reported in the literature (1 to 6) show a fair general agreement which suffices for the recognition of gross abnormalities. For clinical purposes, however, the electrophoretic method will find its full use only when purely technical uncertainties are reduced well below the level of clinical interpretation. This report will give the results obtained with normal subjects, using what appears to be the best present technic (7), and a discussion of various uncertainties encountered in analysis of the data.

TECHNIC

Blood for analyses was taken from healthy young male subjects in a post-absorptive state, except as specifically noted. When anticoagulant was required, potassium oxalate was used. After separation of serum or plasma, a sample was taken for micro Kjeldahl determination (8) of total protein and albumin:globulin ratio according to the Kingsley modification (9) of the Howe sodium sulfate fractionation.

Electrophoretic technic followed a method previously described (7); for detailed description, this paper should be consulted. The buffer system, 0.1 *N* NaV–0.02 *N* H V,[3] giving a pH of 8.6 at 25° C., not only minimizes asymmetry between ascending and descending patterns but also allows the appearance of a previously hidden component, namely, α_1 globulin which falls between albumin and α globulin of the older notation (in Longsworth's notation α_2 globulin). Use of the title, globulin, for the α_1 component is arbitrary and may require later revision when its properties have been thoroughly studied. In addition, a cell of double the usual length is used, thus improving resolution. Figure 1 shows a typical normal pattern.

Plasma or serum, after a preliminary dilution with buffer (1 volume plasma, 2 volumes buffer), was dialyzed against buffer in the cold for 2 or more days. The proteins were caused to migrate in electric fields, averaging about 6.5 volts per cm. for 180 to 200 minutes; under these conditions albumin, the leading component, had shifted 40 to 50 mm. from the original boundary. To allow calculation of mobilities, the current was frequently measured during each run; in addition, the pH and the conductivity of both the protein and buffer solutions were measured.

No general agreement exists as to the best procedure for analyzing the photographed patterns. Difficulty in deciding whether to use the ascending or descending pattern or both, for calculation of concentrations and mobilities of components, arises from the same cause that leads to asymmetry between the patterns, namely, the establishment of small buffer concentration gradients at the boundaries of the protein components. Since the basis for estimating concentrations is the change of refractive index at the boundaries and since a coincident buffer gradient in itself produces a change of refractive index, it may be easily seen that the protein concentration as estimated from the area under any peak in the pattern is indefinite by a small additive term. In the descending pattern, protein components have moved away from the initial boundary position, leaving there a gradient of buffer salt which causes the ϵ peak in this pattern. Migration of proteins proceeds through the initial boundary site in the ascending channel, however; here is developed a larger gradient, known as the δ peak, which includes gradients of proteins as well as of buffer salts. These buffer

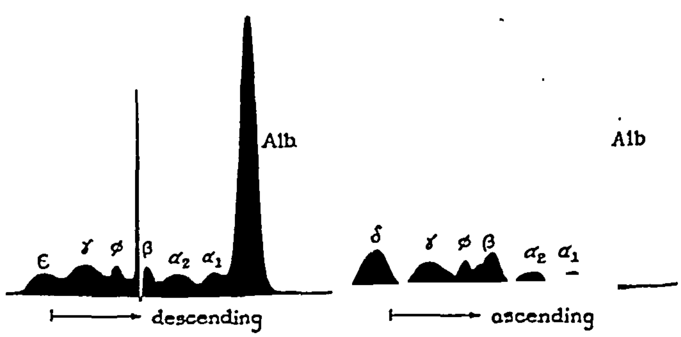

FIG. 1. NORMAL PLASMA PATTERN

[1] The Bureau of Medicine and Surgery does not necessarily undertake to endorse views or opinions which are expressed in this paper.

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[3] V = diethylbarbiturate.

salt gradients, by causing local variations in pH and electric field, introduce uncertainties in the calculation of mobility.

There is as yet no adequate theory that would allow precise account for the effect of salt gradients. Lacking this, one must make various simplifying assumptions (10, 7). If it is assumed that, within the limits of necessary precision, the increments of refractive index due to salt gradients are proportional to the concentrations of proteins at the boundaries and by the same factor for each, these salt effects cancel out in the calculation of the relative proportions of the protein components which may then be determined as ratios of the corresponding areas. Use of the ascending pattern requires the additional assumption that in the δ boundary, the protein components maintain the same relative proportion as in the original specimens. It would perhaps be wiser to avoid the need of this additional assumption by restricting analysis to the descending pattern were it not that in this pattern, uncertainty arises from a disturbance in the region of the β globulin component.

One test of the advisability of using both patterns to estimate proportions of proteins is found in comparison of values obtained from each pattern. Ratios, in each case, of the component area to the total area (exclusive of δ and ϵ boundaries) are calculated; under the above assumptions, they should measure the fraction of the total protein contributed by each component, hence they should be equal for corresponding components in both channels. Both normal and pathological subjects are included in this calculation as the issue is not of normality but of internal consistency in the method. As may be seen (Table I), the ratios of albumin fractions

TABLE I

Comparison of the concentrations estimated for protein components in the ascending and descending patterns

As a preliminary calculation, the fractions of total protein area contributed by the protein components are measured in each pattern. This table shows for each component the ratio of the fraction estimated in the ascending pattern to the corresponding fraction estimated in the descending pattern. Values shown are the means and standard deviations of data calculated from 35 pairs of patterns.

α_1, α_2, β, ϕ, γ denote globulin components, where ϕ is fibrinogen.

Component	Albumin	α_1	α_2	β	ϕ	γ
Mean ratios $\frac{\text{ascending}}{\text{descending}}$	1.00	0.92	1.04	0.88	1.15	1.12
Standard deviation of ratios	±0.050	±0.174	±0.187	±0.124	±0.177	±0.167

TABLE II

Multiple determinations on a single sample of blood

Two migrations were done (serum and plasma) with 6 photographs in each at successive intervals. *Time* denotes minutes from start of migration. *Spread* shows displacement in mm. of ascending albumin peak from initial boundary. Components are designated as in Table I. The last 7 columns show ratios of area of component to total area of pattern (exclusive of δ and ϵ boundaries); values are averages of ascending and descending patterns.

	Time	Spread	$\frac{\text{Alb.}+\alpha_1}{\text{Total}}$	$\frac{\text{Alb.}}{\text{Total}}$	$\frac{\alpha_1}{\text{Total}}$	$\frac{\alpha_2}{\text{Total}}$	$\frac{\beta}{\text{Total}}$	$\frac{\phi}{\text{Total}}$	$\frac{\gamma}{\text{Total}}$
	min.	*mm.*							
Serum	60	16.0	0.639			0.077	0.119	0	0.166
	90	23.0	0.659			0.078	0.111	0	0.154
	120	31.0	0.676	0.632	0.045	0.066	0.108	0	0.151
	150	38.5	0.677	0.634	0.043	0.070	0.112	0	0.143
	180	45.5	0.691	0.643	0.048	0.063	0.105	0	0.141
	210	49.5	0.664	0.618	0.047	0.070	0.110	0	0.156
Average			0.668	0.632	0.046	0.071	0.111	0	0.152
Plasma	60	16.0	0.648						
	90	23.0	0.641			0.073	0.105	0.051	0.132
	120	31.0	0.646	0.602	0.044	0.070	0.102	0.052	0.132
	150	38.0	0.647	0.596	0.051	0.056	0.106	0.057	0.137
	180	44.5	0.651	0.601	0.050	0.067	0.096	0.060	0.128
	210	50.5	0.672	0.621	0.050	0.063	0.092	0.052	0.129
Average			0.651	0.605	0.049	0.066	0.100	0.054	0.132
$\frac{\text{Average serum fraction}}{\text{Average plasma fraction}}$			1.03	1.04	0.94	1.08	1.11		1.25

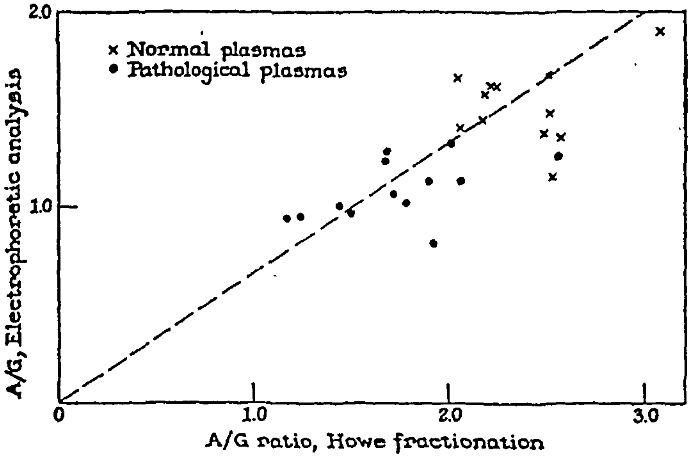

FIG. 2. COMPARISON OF A/G RATIOS ESTIMATED FROM ELECTROPHORETIC ANALYSIS AND FROM NITROGEN DETERMINATION AFTER HOWE FRACTIONATION

were found dispersed about a mean close to unity, indicating that no systematic influence affected this component. In the case of β globulin, however, despite considerable variation it appears that a lower value is calculated from the ascending than the descending pattern; associated with this discrepancy, there must of course be complementary variations in the ratios of other globulin fractions since in both patterns, the sum of the fractions is unity. It thus is found that ratios of β globulin + fibrinogen areas correspond closely, a fact that suggests that some of the difficulty might arise from uncertainty in allocating to each component its proper share of the total area in the graphical measurements. Accordingly, for the purpose of the present paper, the fractions of total protein assigned to components will be defined by the average of fractions calculated from the two patterns.

The problem of proper allocation of areas just encountered is one for which there is no satisfactory answer. It might appear that the ideal method is that of Pedersen (see Longsworth (7)) in which, starting from either end of a pattern, one reflects into the pattern a curve symmetrical about the axis of the peak, subtracts this curve from the ordinate of the pattern and proceeds to the next peak. However, the uncertainty that arises from the dubious assumption of complete symmetry of the peaks, inability to proceed through the β globulin disturbance of the descending pattern, and cumulative graphical errors of the process leave no advantage to this method over the simpler procedure of Tiselius and Kabat (11). In this method, one drops a perpendicular to the base-line from the minima between peaks and assumes that the area for any component lost by overlap into adjacent areas is just offset by contribution of area by adjacent components. The approximation involved in this assumption may be expected to become progressively worse as peaks are crowded together to form a high curve with only shallow valleys separating the peaks.

As a test of the scatter to be expected from variable degrees of resolution, experiments were carried out in which photographs were taken at 30-minute intervals from 60 to 210 minutes after start of migration. For these 2 experiments, blood was taken from one individual, then divided between 2 tubes, one of which contained oxalate and the other no anticoagulant. Data obtained with the plasma and serum are shown in Table II. It is remarkable that even poorly resolved patterns give quite a good estimate of relative proportions.

This experiment also furnishes a test of the difference between serum and plasma since the material was obtained by division of a single sample. As a first approximation, the difference lies in disappearance of the fibrinogen component. If this were the only difference, one would expect that the fractions of total protein contributed by other components would be about 5 per cent greater in serum than in plasma owing to the fact that fibrinogen is present in the plasma in this amount. In the cases of β and γ globulin, the somewhat greater fractions found in the serum could be explained by the probable as-

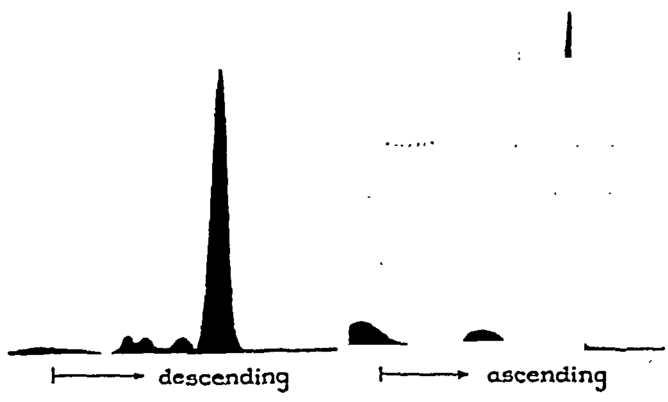

FIG. 3. ELECTROPHORETIC PATTERN OF PROTEINS IN THE "ALBUMIN" FRACTION AFTER SODIUM SULFATE PRECIPITATION

sumption that the narrow fibrinogen peak of the plasma pattern gains more area than it loses by overlap with these adjacent peaks. An actually lower value for the α_1 fraction in serum than in plasma suggests the possibility that some of this component is removed when blood clots. More data are needed to establish the point.

USE OF CHEMICAL DATA

To the clinician, the usefulness of electrophoretic data might be enhanced by converting the ratios into absolute values since a ratio of two concentrations might be varied by change in value of either one or remain constant under proportional change of both. This conversion is accomplished by multiplication of the computed fraction of total protein contributed by various components into the chemically determined protein concentration, giving values for the components in grams per 100 cc. This is, of course, an arbitrary procedure since the various protein components doubtless vary in nitrogen content, but the uncertainties involved in this calculation probably are no greater than others inherent in the method. From data presented in this way, the original electrophoretic ratios are readily recalculated.

Chemical data also afford a comparison between albumin:globulin ratios measured chemically and electrophoretically. As shown in Figure 2, electrophoretic A/G ratios tend to be systematically lower than the chemical values. A fraction measured as globulin by electrophoresis escapes precipitation by sodium sulfate, appears in the filtrate, and is measured as part of the "albumin" determined by Howe's method. This is shown in a pattern obtained with the "albumin" fraction (Figure 3) which remained in solution after the usual sodium sulfate fractionation. The supernatant solution was concentrated in a cellophane bag by alternate drying with an electric fan and dialysis against distilled water before final equilibration against buffer. It will be noted that an appreciable amount of globulin as defined from the electrophoretic patterns remains in solution. As measured, the electrophoretic A/G ratio has a value of approximately 2/3 of the chemical ratio determined on the same specimens of normal or pathological plasma; this is in fair accord with the relation which can be computed from data in the literature on normal and pathological plasmas. Although the scatter of this relation allows only rough calculation, it is possible to make some translation from the large body of data obtained by Howe's method to electrophoretic equivalents.

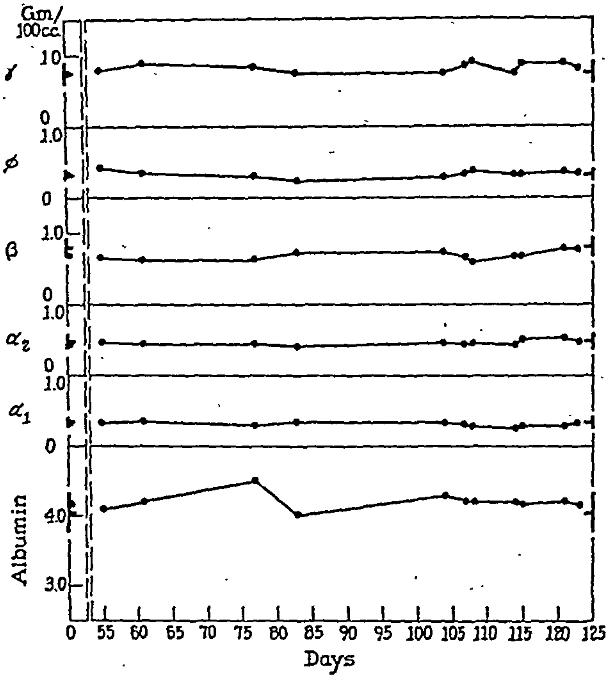

FIG. 4. CONSECUTIVE OBSERVATIONS OF THE PLASMA OF ONE NORMAL INDIVIDUAL

On the ordinates are indicated the mean value and standard deviation for each component.

TABLE III

Comparison of distances migrated in the two channels
(25 normal and pathological plasmas)

Component	Albumin	α_1	α_2	β	ϕ	γ
Ratio of distances migrated ascending/descending	1.06	1.10	1.13	1.12	1.16	1.13
Standard deviation of ratio	±0.011	±0.024	±0.033	±0.050	±0.044	±0.059

Symbols as in Table I.

Mobilities

The apparent mobility of a component is affected by variation in protein concentration. Ideally, the data should be corrected to some standard state, such as infinite protein dilution in a given buffer, but the present knowledge of the salt and pH gradients throughout the cell is

TABLE IV

Normal values (15 normal plasmas)

Components indicated are albumin, α_1, α_2, β globulins, respectively, fibrinogen and γ globulin. A/G denotes albumin globulin ratio. Concentrations are given as r (ratio of component area to total area exclusive of δ and ϵ peaks) and as grams per 100 cc.

		Albumin		α_1		α_2		β		ϕ		γ		A/G
		r	*grams per 100 cc.*	r	*grams per 100 cc.*	r	*grams per 100 cc.*	r	*grams per 100 cc.*	r	*grams per 100 cc.*	r	*grams per 100 cc.*	
Concentrations	Mean	0.603	4.04	0.046	0.31	0.072	0.48	0.121	0.81	0.051	0.34	0.110	0.74	1.53
	Standard deviation	0.028	0.27	0.007	0.051	0.013	0.083	0.019	0.126	0.006	0.059	0.025	0.151	0.181
Mobilities (cm^2/volt sec. $\times 10^5$)	Mean	5.94		5.07		4.08		2.83		2.14		1.02		
	Standard deviation	0.267		0.236		0.256		0.241		0.252		0.282		

too limited to allow this to be done without ambiguity. An alternative procedure described below appears to be a compromise adequate for the present purposes.

One protein component migrates in the original protein mixture, namely, albumin in the descending channel. Others, however, migrate in regions from which various components are missing since, in either channel, a protein component is present only below its boundaries. Thus, when a mixture is partially separated by electrophoresis, one would find in proceeding from pure buffer in one channel to pure buffer in the other, a stepwise increase of total protein concentration to a maximum of complete mixture at the center, then a complementary stepwise decrease on the other side. In the central region where all components are present, there is a region of protein and buffer gradients, the δ boundary, near the initial boundary position of the ascending channel. At all these steps the associated buffer gradients are of unknown magnitude but it would appear reasonable to assume that in the two channels, the globulin components are affected by oppositely directed influences. While albumin mobility was calculated from the velocity of the descending peak, therefore, globulin mobilities were based on average velocities in the two channels with pH and conductivity data of the original protein mixture.

An estimate of the discrepancies in the data from the two channels may be made from the data in Table III in which are given the ratios of migration distance for each component in ascending and descending patterns. Presumably the buffer gradients at the boundaries of globulin components are small; if this be assumed, it follows that the 6 per cent increment found for albumin is due to the δ boundary gradient. Increments for the globulins are larger, however. From this fact it is seen that the lengths of columns of globulins from their descending to their ascending boundaries are greater than the length of the albumin column. They must, therefore, either experience a greater relative concentration change than albumin through the δ boundary or there must be appreciable changes in globulin concentrations at the descending boundary of the albumin component. Either of these possibilities unsettles the calculation of protein concentrations, so that with this, as with mobility, the value of the data is comparative.

Purely graphical errors arise from the fact that the distance of migration should be measured from the initial boundary to the centroidal ordinate of a peak as it would appear in the absence of overlapping peaks. Because of the overlap of peaks, however, their true contours are unknown; distances were therefore determined using the maxima of the peaks. The systematic errors from this cause appear to be quite constant.

Observations on the plasma of a single individual over a period of time

For some purposes, more interest will attach to the changes in successive electrophoretic analyses using individual patients than to group

averages of patients of a given disease type. Only one normal individual has been thus followed in consecutive observations (Figure 4). While this single series is not adequate to define the extent of normal fluctuation with time, it indicates a reasonable stability of the protein pattern. The causes of the small physiological variations found are at present not known. More variation is present than would be expected from technical errors in analysis.

Normal standards

In Table IV are given the values derived from the analysis of 15 plasmas, taken from normal young male adults, 4 or more hours after meals.

SUMMARY

Normal values for the concentrations and mobilities of protein components in human plasma have been determined by electrophoresis at pH 8.6 in sodium veronal buffer. Albumin: globulin ratios, measured electrophoretically, were found to be roughly two thirds the ratio found by chemical fractionation of the same sample.

Various sources of technical uncertainty are discussed.

It is a pleasure to acknowledge the guidance of Drs. T. Shedlovsky, L. G. Longsworth, and D. A. MacInnes.

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F_1 AND F_2 OF NAJJAR AND HOLT IN THE URINE OF NORMAL YOUNG MEN [1]

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(Received for publication January 13, 1944)

Najjar and Holt (1, 2) have demonstrated that their fluorescent urinary pigments, F_1 and F_2, are related to nicotinic acid metabolism in such a way that their estimation in urine is of diagnostic use in human pellagra (1) and in canine black tongue (1, 2). As nicotinic acid deficiency progresses, the daily excretion of F_1, starting from a low level, slowly rises and then falls again; and the daily excretion of F_2, starting from a normal level, falls progressively to zero. Vigorous treatment of pellagrins with nicotinamide causes, within 3 days, a large increase in F_2 and a decrease in F_1. Extending this work, Holt and Najjar (3, 4, 5) proposed the routine measurement of urinary F_2 in nutritional surveys as an indication of the nicotinic acid content of the body. They state that when F_2 is absent in urine collected in the post-absorptive state,[3] the patient's stores of nicotinic acid are low. Oral administration of nicotinamide to normal subjects in a good nutritional state leads to a prompt increase in F_2, maximal in 2 to 4 hours and subsiding to normal within 6 hours. Coulson and his coworkers (6) have also made a detailed study of the effects of various nicotinic acid derivatives on urinary F_2 estimations. Their results are much the same as those of Najjar and Holt.

In developing suitable rapid field methods for assessing the nutritional status of young men (7), we have investigated the conditions under which the estimation of urinary F_1 and F_2 might be expected to yield useful information. In particular, we sought to answer 3 specific questions: first, whether the level of these pigments in urine in a post-absorptive state agrees with the criteria of Holt (3); second, whether their excretion following administration of nicotinamide correlates with other clinical data; and third, whether their excretion following the administration of various mixtures of vitamins has the same significance as after nicotinamide alone. Our observations have been limited solely to normal young men, and we have not studied pellagrins.

EXPERIMENTAL

We will discuss first, analytical methods; second, the stability of F_2 in urine; and third, the F_1 and F_2 elimination of young men under a variety of conditions.

Analytical methods

All estimations of F_1 and F_2 reported in this paper were carried out by the method of Najjar and Wood (8) or by a modification of this method for rapid field use to be described in a forthcoming paper (7). This modification is different from the method of Huff and Perlzweig (9). In our determination, fluorometry was either photoelectric or visual and the reference standards were either quinine or thiochrome.

Stability of F_2 in urine

Field surveys sometimes necessitate the collection of specimens in climatic extremes, transportation over considerable distances, and storage for various lengths of time before analysis. The stability of F_2 under simulated field conditions was therefore investigated. Random samples of urine were collected from several normal

[1] The present work was financed in part under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the President and Fellows of Harvard College. We acknowledge substantial financial support from the Corn Industries Research Foundation.

[2] We are indebted to the 1st Service Command and the Quartermaster Corps, U. S. Army, for placing Cpl. M. Castiglione, Technician Fifth Grade, on detached duty in this laboratory.

[3] The term "post-absorptive" is used in the sense of Benedict. A subject is in the post-absorptive state when he is resting 14 hours or more after his evening meal and after a night's sleep. In other words, he is fasting before breakfast.

men and from several men who had taken orally 100 mgm. of nicotinamide, 12 hours before collection. In each case, the specimens were pooled, acidified to approximately pH 4 with glacial acetic acid, and some portions were stored in an ice-box at 10° C. and others in an incubator at 50° C.

Table I summarizes a typical experiment. (Throughout this paper 3 arbitrary conventions in terminology will be followed: first, the expression "γ" represents the amount of F_1 or F_2 in quinine units; second, the term "normal urine" refers to urine collected from men on a good normal diet, not supplemented by vitamin preparations; and third, the term "loaded urine" refers to urine collected from men who had within the previous 12 hours ingested one or another mixture of vitamins.) This table brings out 4 points. First, within 6 days, F_2 in normal urine showed no significant change at either temperature. Second, within 2 days of storage, F_2 in loaded urine increased significantly at both temperatures, the increase continuing for at least 6 days. Third, the increase in F_2 in loaded urine was not affected by changes of temperature. Fourth, the increase was not prevented by thymol, indicating that bacterial growth was not the cause of the increase.

The stability of F_2 has been discussed by Najjar and Wood (8), who found that the pigment is destroyed slowly in air and more rapidly in the presence of alkali and potassium ferricyanide. In the light of the recent studies on the relation between F_2 and N-methylnicotinamide (10 to 13), a reasonable hypothesis to explain our results is that in normal urine no precursor of N-methylnicotinamide is present, but in loaded urine, such a precursor is present in significant amounts and breaks down by some mechanism other than bacterial action. The practical conclusions of our studies on stability are: first, insofar as F_2 is concerned, normal urine may be transported safely, stored, and analyzed at leisure, provided the pH is lower than 4; and second, that loaded urine should be handled reasonably promptly.

TABLE I

Stability of F_2 in urine

Storage tempera-	Sample	Days exposed			
		0	2	4	6
°C.		γ per cent			
10	Normal urine	1	1	1	1
10	Loaded urine	14	22	19	28
10	Loaded urine + thymol	14	24	19	31
50	Normal urine	1	1	1	1
50	Loaded urine	14	24	24	31
50	Loaded urine + thymol	14	31	24	31

F_1 and F_2 elimination under a variety of conditions

In the succeeding paragraphs, 4 points will be brought out. First, with certain important reservations, our findings substantiate in general the statements made by Holt (3) concerning the significance of F_2 in post-absorptive urine. Second, responses to identical oral doses of nicotinamide vary among subjects. This variability tends to invalidate the use of F_2 alone as a criterion in nicotinamide tolerance tests. Third, F_1 excretion is probably influenced by the dietary level of thiamine. Finally, as would be expected from points 2 and 3, the interpretation of the changes in F_1 and F_2 after the administration of various mixtures of vitamins is much complicated by individual idiosyncracies in the metabolism of nicotinic acid and also by the level of vitamins in the subject's diet.

(*1*) *F_1 and F_2 in post-absorptive urine.* Samples of urine were obtained over 1- to 3-hour periods from 169 normal young men in the post-absorptive state. Of these, 20 were subsisting in New England on an adequate civilian diet and 149 were existing in the Mojave Desert on a diet containing over 100 grams of protein daily, with egg, milk, and meat products at every meal. In none of these 169 men were any signs or symptoms of nicotinic acid deficiency seen.

In the 169 specimens of urine, the average hourly excretion of F_1 was 0.2γ, and it was zero in 95 cases (see Table V). Therefore, the presence or absence of F_1 in the fasting urine bears no simple relation to the amount of nicotinic acid in the tissues or in the diet.

The average hourly excretion of F_2 was 2.0γ and in 6 cases was zero (see Table V). These results should be compared with Holt's statement (3): "The quantity of . . . F_2 found in the

test specimen indicates the extent of the body reserves of . . . nicotinic acid. As long as any . . . F_2 is found in the test specimen this indicates . . . that deficiency . . . is not to be feared. A zero excretion . . . indicates, however, that . . . such an individual is potentially deficient." In our group of 169 men, whose intake of nicotinic acid was unquestionably adequate, the F_2 excretion in the post-absorptive state was in reasonably good agreement with the dietary and medical data. However, 6 cases were found whose F_2 excretion was zero without any other evidence of nicotinic acid deficiency. Holt's criterion, therefore, is possibly too strict, and before a diagnosis of early nicotinic acid deficiency can be made, other dietary and medical evidences should substantiate a zero F_2 in the post-absorptive urine.

In contrast to the above findings on normal young men, we found in one case that the presence of F_2 in considerable amounts is compatible, at least for short periods of time, with complete nutritional deficiency. Table II contains data on this one man. During 16 days of voluntary fasting, no F_1 appeared, riboflavin fluctuated erratically, thiamine and ascorbic acid decreased steadily, and F_2 increased tenfold. In this case, the levels of thiamine and vitamin C changed with the patient's known nutritional state, but the F_2, if considered alone, would have led to the fallacious conclusion that he was well supplied with nicotinic acid.

TABLE II

Changes in urinary vitamins during a voluntary complete fast

Period of fast	Substance in urine				
	F_1	F_2	B_1*	B_2**	C***
day	*γ per 24 hours*		*γ per 24 hours*		*mgm. per 24 hours*
1	0	3	104	1040	
4	0	2	83	990	75
6	0	30	74	1225	44
8	0	34	66	1753	0
16	0	30	25	990	0

* Analysis by method of Egaña and Meiklejohn (14).
** Analysis by method of Najjar (15).
*** Analysis by method of Mindlin and Butler (16).

(2) *Individual responses to oral doses of nicotinamide.* During an investigation of the reliability of the oral nicotinamide tolerance test, we found that individual responses were varied. This variability is illustrated in Table III. Three normal young men, subsisting on adequate diets, received 50 mgm. of nicotinamide orally at each meal during a period of 2 or 3 days. F_2 was estimated in specimens of urine collected in the post-absorptive state as well as during the rest of the day. The table shows 2 points. First, one subject, P. R., showed a large response in both specimens of urine; another, F. C., no response; and third, F. S., a moderate response. Second, in one case, P. R., the fasting hour specimen showed the greater response, and in another, F. S., the 24-hour specimen. It is concluded that this wide individual variation among men known to be adequately supplied with nicotinic acid indicated that F_2 alone cannot be used as an accurate estimate of the effects of ingestion of nicotinamide.

TABLE III

Individual responses to oral doses of nicotinamide

Subject	Period	F_2 in urine, Average 2 hour fasting	F_2 in urine, Total in 24 hours
		γ per hour	
P. R.	Before	12	12
	2 days' loading*	126	28
	After	2	
F. C.	Before	8	8
	3 days' loading*	6	6
	After	9	4
F. S.	Before	5	3
	3 days' loading*	7	14
	After	4	

* All subjects took 50 mgm. of nicotinamide by mouth at each meal.

(3) *Effect of dietary level of thiamine on F_1 and F_2 excretion.* In tolerance tests employing mixtures of vitamins, we frequently found large amounts of F_1 in the urine. The effect of thiamine on the excretion of F_1 and F_2 was therefore investigated. The same 3 subjects described in the preceding paragraph ingested 5 mgm. of thiamine hydrochloride at each meal for 3 days. Specimens of urine were collected as in the nicotinamide tolerance tests described above. Table IV shows the results. Particular attention may be called to 2 points. First, in 1 subject, P. R., there was a significant increase in F_1 following thiamine ingestion, and in the other 2, there was

no significant increase. All subjects showed a small increase in F_2. It is concluded from these observations that in some individuals, the interpretation of urinary levels of F_1 and F_2 may be complicated by this possible effect of thiamine.

TABLE IV

Individual responses of F_1 and F_2 in mgm. per hour following ingestion of thiamine

Subject	Period	Average F_1 2-hour fasting	Average F_1 Total 24 hours	Average F_2 2 hour fasting	Average F_2 Total 24 hours
		γ per hour		γ per hour	
P. R.	Preloading	5	2	7	3
	2 days' loading*	12	39	7	9
	Post-loading	13	7	12	12
F. C.	Preloading	12	13	4	4
	3 days' loading*	6	11	6	10
	Post-loading	16	9	8	8
F. S.	Preloading	0	0	5	4
	3 days' loading*	0	0	6	7
	Post-loading	0	0	5	3

* All subjects took 5 mgm. thiamine hydrochloride at each meal.

(4) *F_1 and F_2 excretion following ingestion of various mixtures of vitamins.* In field surveys of nutritional status, it is common to perform tolerance tests with mixtures of vitamins. In the course of running such tests, we have obtained data following single administrations, and following administrations daily for 8 weeks.

Table V shows the variability in response of F_1 and F_2 among the 169 men described above whose nicotinic acid intake was unquestionably adequate. We have previously discussed F_1 and F_2 in their post-absorptive urine. We shall now consider F_1 and F_2 separately in the case of their loaded urine.

An increase in F_1 following the test dose was seen in about 10 per cent of the subjects. This increase was possibly due to thiamine as described above. We may conclude that in field surveys little information is to be gained by estimating F_1 in tolerance tests.

An average hourly increase of about tenfold was seen in the F_2 elimination following the test dose. Nevertheless, about 15 per cent of the men failed to respond. We draw 2 conclusions with respect to F_2 in tolerance tests. First, a high F_2 following the test dose is significant and probably means adequate saturation of the body with nicotinic acid. Second, a low F_2 must be interpreted with caution with due allowances for variability and with careful consideration of other dietary and medical data.

Very considerable increases in F_1 and F_2 may be observed following ingestion of large doses of vitamins over prolonged periods. In Table VI, urinary data are presented for groups of subjects subsisting for 8 weeks on normal diets, on diets low and high in protein, and on diets devoid of ascorbic acid. All subjects received a daily supplement of brewers' yeast extract,[4] containing in each dose 5 mgm. of thiamine, 2 mgm. of ribo-

[4] Standard Brands, Inc., kindly donated the Type III extract of brewers yeast which we used in part of this study.

TABLE V

Excretion of F_1 and F_2 before and during 4 hours following oral doses of vitamins *

Group of subjects	Number of subjects		F_1 Fasting urine	F_1 Total excess in 4 hours	F_2 Fasting urine	F_2 Total excess in 4 hours
			γ per hour		γ per hour	
New England	20	Mean	0.1	1	1.2	75
		Range	0 to 0.7	0 to 9	0 to 3	4 to 221
		Percentage of men showing increase**		10		100
Mojave Desert	149	Mean	0.3	2.3	2.4	58
		Range	0 to 3	0 to 136	0 to 20	0 to 844
		Percentage of men showing increase**		8		81

* The test dose was an aqueous solution containing 5 mgm. thiamine hydrochloride, 5 mgm. riboflavin, 50 mgm. nicotinamide, and 500 mgm. ascorbic acid.

** "Percentage showing increase" means the percentage of subjects who excreted more F_1 and F_2 per hour following the dose than before.

TABLE VI

*Excretion of F_1 and F_2 after supplementation with yeast for 8 weeks**

Type of diet	Number of subjects	F_1		F_2	
		Mean	Range	Mean	Range
		γ per day		*γ per day*	
Normal	7	21	0 to 60	27	7 to 39
Low protein	6	15	0 to 40	33	7 to 56
High protein	8	32	0 to 74	34	1 to 55
Vit. C free	14	21	0 to 88	33	10 to 61

* Each subject received one daily dose of Fleischman's Type III Brewers' Yeast Extract containing 5 mgm. thiamine, 2 mgm. riboflavin, and 11.5 mgm. of niacin.

flavin, and 11.5 mgm. of niacin. The average excretion of F_1 and F_2 per day was high in almost every case, the average F_2 excretion being the same as that of the fasting man after 6 days. These results emphasize again the necessity of interpreting urinary data after consideration of other clinical data.

COMMENT

In considering the practical utility of estimating F_1 and F_2 in nutritional surveys, the 2 substances should be discussed separately.

The presence or absence of F_1 in the urine of normal subjects, either in the post-absorptive state or after test doses of vitamin, appears to bear little, if any, relation to their nutritional state. We must emphasize that this conclusion does not necessarily apply to pellagrins.

The interpretation of the levels of F_2 in the urine is complicated by the wide variety of individual responses to the dietary level of nicotinic acid. Several possible causes for this variability present themselves at once, some more likely than others. First, individual idiosyncrasies of absorption, although possible, appear to be unlikely as the chief cause of variation. In our series, wide individual variations were present even after 8 weeks of supplementation of diets already rich in animal protein. Equally wide variations were found after only 2 or 3 days of supplementation. Second, different responses at different stages of chemical unsaturation may be responsible for a certain percentage of cases showing anomalous responses. This phenomenon has been shown by Najjar and Holt (1, 2) in canine black tongue and in human pellagra and appeared to be operative in our subject who fasted for 16 days. This cause can hardly play an important rôle in young men on normal diets. Third, the most reasonable hypothesis seems to be that the end-products of nicotinic acid metabolism occur in urine in many forms and in different proportions in different subjects. Ingested nicotinamide is known to give rise in the urine to nicotinic acid, nicotinamide, nicotinuric acid, and N-methylnicotinamide (the precursor of F_2) (17). For unexplained reasons, one or the other of these substances sometimes predominates, and some may not appear at all (17). Convincing data on this multiplicity and variability of nicotinic acid intermediaries have been presented by Sarett, Huff, and Perlzweig (17). One mechanism has been elucidated by Perlzweig and coworkers (18), who showed that the aerobic methylation of nicotinamide by slices of rat liver is sometimes, but not always, enhanced by methionine.

In view of evidence such as the above, it would appear desirable to estimate as many as possible of the urinary nicotinic acid derivatives in assessing the status of the body's stores of the vitamin. At present, this is a laborious task, even in a well-equipped laboratory. For field surveys on normal young men, it appears that F_2 is the easiest and quickest single substance to assay. Data on it must, however, be interpreted with caution, after careful correlation with other independent evidence and with full realization of the limitations of the findings.

SUMMARY

1. An investigation has been made into the utility of routine estimations of the fluorescent urinary pigments, F_1 and F_2 of Najjar and Holt, in nutritional surveys of normal young men.

2. F_2 in specimens of urine obtained in the post-absorptive state is stable for at least a week at 50° C. Under the same conditions, F_2 in urine after an oral dose of nicotinamide increases independently of bacterial action.

3. The following conclusions are drawn concerning the urinary level of F_1: (*a*) the presence or absence of F_1 in the urine of normal young men, either in the post-absorptive state or after test doses of vitamins, bears little or no relation

to nutritional state; (*b*) in some subjects, it was discovered that ingestion of thiamine appears to increase the excretion of F_1.

4. The following conclusions are drawn concerning the urinary level of F_2 in the post-absorptive state: (*a*) the level of F_2 in the urine of normal young men correlates reasonably well with other dietary and clinical evidence concerning their nicotinic acid stores; (*b*) however, a small percentage of men known to be eating a diet adequate in nicotinic acid normally excrete no F_2 in the urine; and (*c*) the urinary F_2 of a man who fasted for 16 days reached high levels.

5. The following conclusions are drawn concerning the level of F_2 in urine after test doses of vitamins: (*a*) the usual response of normal men to test doses of nicotinamide is to increase the excretion of F_2; (*b*) a small percentage of men known to be adequately supplied with dietary nicotinic acid shows no increase in F_2 after test doses; (*c*) the level of F_2 must be interpreted with caution, with due allowance for variability and careful consideration of other dietary and medical data.

6. A reasonable explanation for the extreme variability of urinary F_2 is presented. There is known to be a multiplicity of urinary intermediaries in nicotinic acid metabolism, of which F_2 is only one. For unexplained reasons, one or the other may predominate at the expense of the rest, and some may not appear at all after test doses of nicotinamide. Hence, as many as possible of these intermediaries should be estimated when nicotinic acid stores are being assessed.

6. For field studies, F_2 is the easiest derivative of nicotinic acid to estimate. In our experience, its estimation in post-absorptive urine and in the urine following test doses of nicotinamide yields useful information. However, caution must be used in interpretation.

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TRAUMATIC SHOCK. VII. A STUDY OF THE PROBLEM OF THE "LOST PLASMA" IN HEMORRHAGIC, TOURNIQUET, AND BURN SHOCK BY THE USE OF RADIOACTIVE IODO-PLASMA PROTEIN[1]

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In paper IV of this series (1), evidence was given that there was no significant loss of plasma into the tissues of the dog in untreated fatal shock from hemorrhage. The technic utilized involved the tagging of plasma proteins by radioactive sulfur or radioactive bromine. Before extending the observations to shock in which there is an area of local injury, such as occurs in tourniquet and burn shock, an attempt was made to reduce the errors inherent in the radioactive technic (1 to 4). These errors center in (*1*) the problem of obtaining the lowest possible halogen composition of the tagged protein in order to reduce denaturation of the protein to a minimum; (*2*) methods of determining the total intravascular plasma protein content of the tissues to be analyzed. This requires as accurate an estimate as can be made of the red cell fraction of the capillary blood. For this purpose, in addition to hemoglobin determinations of perhaps somewhat doubtful accuracy, estimates of the capillary hematocrit have been utilized. (*3*) The preparation of tissues for analysis so as to permit maximum recovery of the radioactive element. In preparing tissues containing radioactive bromo-protein for radioactivity measurements, an oxidative method of isolating the halogen was used. In the oxidation procedure, there was a possibility of loss of some bromine by volatilization in spite of the presence of $AgNO_3$. Further, the excess bromide added as a carrier, together with the chloride also present, produced with silver nitrate a precipitate of sufficiently variable thickness and particle size to affect by self-absorption the accuracy of the radioactivity readings. There is also a possibility of some loss in the handling of the precipitates.

In respect to the first category of errors, a constant halogen composition of plasma proteins could be achieved by coupling the protein with a minimum of halogen, which is desirable for approximating the slowest possible rate of disappearance, *i.e.*, the rate of disappearance of normal plasma protein from the blood of the normal dog (1). For this purpose, radioactive iodine proved more satisfactory than radioactive bromine.[2] Hence, all experiments in this communication were performed with radioactive iodine exclusively.

In regard to the second group of errors, the hemoglobin determinations were probably not completely reliable and capillary hematocrits were only estimated. Nevertheless, on the basis of certain considerations to be described under "method," the probable error of these deficiencies was reduced to a minimum and, we have reason to believe, is not great enough to substantially affect the final results. As for the third group of errors, the oxidative method of isolating the radioactive halogen was abandoned and a method adopted involving evaporation to dryness (in the presence of alkali). The technic, described below, reduced the error of tissue analysis for radioactivity from 25 per cent to 10 per cent or less (compare Table I of this communication with Tables II and III of Paper III) (2).

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[2] Radio-iodine, prepared by the transmutation of tellurium, is obtained in arbitrarily high specific activity to which a constant minimum quantity (5 mgm.) of carrier iodine is added for convenience in handling. On the other hand, radiobromine, which is prepared by neutron bombardment of large quantities of ethyl bromide, is unavoidably isolated with variable, but usually large quantities (300 mgm. or more) of non-radioactive bromine from hydrolysis of ethyl bromide.

This communication provides data on the movement of plasma protein (and plasma) [3] from the blood stream into the tissues of the normal dog and of the dog shocked by hemorrhage, a tourniquet, or a burn. The well-known observation that dogs in irreversible shock show wet or hemorrhagic tissues if treated by infusions, particularly of saline, required a special study of the state of capillary permeability in these circumstances. The data to be presented agree with those already reported, to the effect that there is no evidence to substantiate the common belief that a progressively increasing loss of plasma protein and plasma from the generalized capillary bed occurs in shock. Loss occurs only into areas of injury such as produced by a tourniquet or burn. Some of the data and conclusions from this work are already reported elsewhere (5).

METHOD

All dogs received morphine sulfate, 2.0 mgm. per kgm. body weight. Larger doses were administered before the application of a tourniquet. Burns were produced under ether anesthesia. Barbiturates were not used, since the resistance of dogs to shock procedures was found to be markedly reduced by even small doses of nembutal (see experiment I–5).

Hemorrhagic shock was induced as described previously (1). Normal dogs served as controls for the dogs in shock. When we studied the possibility of abnormal escape of plasma protein in the stages of shock which do not respond to transfusion, *i.e.*, "irreversible shock," a dog in shock which did respond to a transfusion served as the control. Radioprotein was injected with the transfusion or with subsequent saline infusions.

Tourniquet shock was induced by applying 5 to 6 turns of heavy-walled rubber tubing as high on the leg as possible, as tightly as possible, and held with a screw clamp. Previous investigators who employed barbiturate anesthesia produced fatal shock if a tourniquet was applied for 5 hours. In our experiments, in which barbiturate anesthesia was not used, fatal shock did not occur unless the tourniquet (one leg) was left on at least 8 to 9 hours. Normal dogs were used as controls. Radioactive protein was injected one-half hour before the tourniquet was removed.

Burns were produced by immersion of 1 or 2 extremities in hot water (98° C.) for varying periods of time. In contrast to the observations of others (6), who used barbiturate anesthesia, we did not produce fatal shock by immersion in water at 98° C. for 20 seconds. In one experiment (I–5, Table V), immersion of 2 legs in water at 98° C. for 45 seconds did not produce fatal shock, but administration of a small amount of nembutal intraperitoneally, 24 hours later, produced a drop in blood pressure to 30 mm. Hg followed by death. For this reason, burns for longer periods of time were used. In such cases, marked hemoglobinemia was produced. Normal dogs were used as controls. In one experiment (I–8), a dog receiving a non-fatal burn was used as a control for a dog with fatal burn shock. Radioactive protein was injected one-half hour before the animal was burned (except in experiment I–8).

PREPARATION OF RADIOACTIVE IODO-PLASMA PROTEIN

A target of cobalt telluride, having received deuteron bombardment for 400 microampere hours, was filed from its copper base and transferred to an all glass distillation apparatus. A desired amount of carrier iodine (2 to 5 mgm.) was added as a potassium iodide solution (1.0 ml. containing 5.0 mgm. of iodine) followed by 20 ml. of 30 per cent nitric acid. After the evolution of the nitrogen peroxide had ceased, iodine was rapidly distilled into cold carbon tetrachloride (25 ml.) and a few ml. of water. At the end of the distillation, the water was run out of the condenser, and the steam was allowed to bubble through the carbon tetrachloride for a few seconds to sweep out all iodine from the condenser. The carbon tetrachloride solution was then washed once with a few ml. of water.

The carbon tetrachloride solution of iodine was then added with swirling in the cold to 50 ml. of plasma (previously extracted once with carbon tetrachloride) or to 20 ml. of a 10 per cent solution of crystallized bovine albumin, followed by 10 ml. of 25 per cent sodium carbonate solution.[4] The violet color of the carbon tetrachloride solution was rapidly discharged on shaking. The mixture was centrifuged and the plasma separated and dialyzed

[3] The capillary leakage hypothesis assumes that plasma (or some of its constituents) escapes from the general capillary bed at a greater than normal rate and that it fails to return to the circulation as fast as it leaves, resulting in a net gain of plasma to tissues outside of localized areas of injury. Since the net loss of the non-protein fraction of plasma without a simultaneous net loss of some protein from the circulation will not produce shock (except in the case of shock from extreme and rapid dehydration), a determination of the presence or absence of a net gain of plasma protein to the extravascular portion of the tissues outside of areas of injury should decide whether or not this hypothesis is tenable. In the subsequent discussion, therefore, while the data obtained refer only to protein shifts, we shall not distinguish between plasma protein loss and whole plasma loss when referring to the concept of leakage due to a generalized increase in capillary permeability. For the purpose at hand, it is not so necessary to demonstrate the existence of an increase in capillary permeability as it is to observe the consequences of such an increase.

[4] The undesirability of using stronger alkali was discussed in a previous publication (1).

against cold running tap water for 36 to 48 hours. The percentage of radio-iodine incorporated in the plasma protein was 15 per cent of the amount used. When 5.0 mgm. of iodine were used as a carrier and about 50 ml. of plasma iodinated, the percentage of iodine in the iodoprotein was about 0.03 per cent. If one molecule of iodine had been incorporated per protein molecule, the plasma protein would have contained about 0.2 per cent of iodine, which is the theoretical upper limit[5] of iodine content for obtaining the slowest possible rate of disappearance of a halogenated protein from the circulating plasma (see previous discussion (1) in the case of bromine). By the time tissue radioactivity measurements were made (5 to 10 days after the bombardment), most of the I^{130} (half life, 13 hours) had disintegrated, and the radiation was essentially that of I^{131} (half life, 8 days). The latter constitutes about 10 per cent of the total original radiation.

STABILITY OF THE IODINE LINKAGE IN IODINATED PLASMA PROTEIN

Following 40 hours of dialysis, about 2 per cent of the total radioactivity was found in the non-protein fraction after precipitation of the protein with trichloroacetic acid. Trypsin hydrolysis at 37° C. for 6 hours at pH 8.0 released 4.0 per cent of the radioactive iodine content of the protein into the non-protein fraction. Acid hydrolysis with 25 per cent sulfuric acid at 90° C. for 6 hours resulted in 40 per cent of the total radioactivity in the non-protein fraction. The stability of the iodine linkage is similar to the bromine linkage, referred to in a previous publication (1), in alkaline enzymatic hydrolysis, but much weaker in strong acid. Previous workers (7) have shown that small amounts of ionic radio-iodine are immediately removed from the blood stream by the thyroid gland. That no great increase in ionic iodine occurred *in vivo* can be inferred from the fact that the thyroid content of radioactive iodine in a normal dog did not rise during a 12-hour period above 0.1 per cent of the radioactive iodine injected as iodoprotein.

PREPARATION OF PLASMA AND TISSUES CONTAINING RADIOACTIVE IODINE FOR RADIOACTIVITY ANALYSIS

Heparinized plasma (1.0 ml.) was evaporated to dryness in small brass cups at about 80° C. Tissues (4.0 grams) were hydrolyzed[6] by heating at 80 to 90° C. in an oven with 25 ml. of IN NaOH in 50 ml. beakers covered with a watch glass. After the tissues had dissolved, the watch glass was removed and concentration allowed to proceed to 10 to 15 ml. The solution was transferred to a graduate and made up to 20 ml. with water. After thorough mixing, 5.0 ml. were pipetted to a glass cup and evaporated to dryness. Because of the hygroscopic nature of the dry alkali, the cups were re-evaporated on the following day and kept in pill boxes until just before radioactivity measurements were made. Dry weights so prepared varied from 600 to 800 mgm., but the dry weights of similar organs in different dogs agreed within 100 mgm. The dry weight per sq. cm. of exposed surface was about 80 to 90 mgm. (glass cups were 34 mm. in diameter).

Greater ease and accuracy in pipetting the solutions was obtained when lean animals were used, since excessive fat in some tissues (especially skin) resulted in soap formation with marked increase in viscosity. When the heating was kept to a minimum, it was possible to dissolve all tissue constituents except fat. Since the fat floated as an oil and contained none of the iodine, it was partially separated from the aqueous solution and discarded.

In order to correlate measurements of plasma (1.0 ml. in brass cups) with those of tissues (1.0 gram in 5 ml. glass cups) for subsequent calculations, 2 standards from the radioactive protein used for injection were prepared by dilution 50 times with dog plasma. One ml. was treated as for plasma, and 0.1 ml. was evaporated in a glass cup with 5 to 6 ml. of dog plasma and 0.5 ml. of 10N NaOH. In this way, the same order of activity and dry weight was approximated.

In the experiment in which red cells containing radioactive iron were used, it was necessary to prepare standards from which corrections could be made for the radioactivity of tissues contributed by the red cell content. To 2 glass cups containing 0.5 ml. of 10N NaOH and 5 to 6 ml. of dog plasma were added (*A*) 0.1 ml. arterial blood (taken at the time of death) and (*B*) $0.1 - \frac{\text{arterial hematocrit}}{10}$ ml. of plasma (taken at the time of death). The measurement of $A - B$ gave the activity of $\frac{\text{arterial hematocrit}}{10}$ ml. of red blood cells. From this, the activity of tissue due to a measured volume of radioactive cells could be calculated. It was always very small in comparison to the activity of iodoprotein.

MEASUREMENT OF RADIOACTIVITY

Radioactivity measurements were made with an electroscope. The modifications incorporated for measuring radioactive sulfur and bromine (2) were extended for radioactive iodine so as to take a 5.0 ml. capacity glass cup referred to above. The use of the larger cup was necessary for measuring the activity in 1.0 gram of tissue, since hydrolyzed tissue required 5.0 ml. of solution per gram for accuracy in pipetting. Had smaller amounts of tissue been measured, more radioactive iodoprotein would have been required for injection. The sliding bar of the electroscope (2) was enlarged to take the larger cup, and a shallow socket was made in the center of the floor of the

[5] By keeping the iodine content well below this upper limit, the chances of a given protein molecule coupling with more than one iodine molecule were reduced to a minimum.

[6] Oxidation of tissues with nitric acid in the presence of silver nitrate, as described for radioactive bromine experiments (1, 2), resulted in volatilization of large amounts of iodine.

TABLE I

*Comparison of measurements of 1.0 gram samples of liver to which were added various dilutions of radioactive iodoprotein**

Dilutions	Radioactivity	Deviation from linearity	Ratio of radioactivity to background
	divisions per second	*per cent*	
1	0.00303	− 1	56
2	0.00150	− 2	28
4	0.000768	0	14
8	0.000368	− 4	7
1	0.000505	0	9
3	0.000188	+10	3
9	0.0000632	+11	1
Background	0.0000532		

* Error was greater when radioactivity was nearer background.

TABLE II

Comparison of measurements of 1.0 gram samples of various tissues to each of which was added a constant amount of radioactive iodoprotein

Tissue	Radio-activity	Devia-tion from linearity	Dry residue weight	Radio-activity	Devia-tion from linearity	Dry residue weight
	divisions per second	*per cent*	*mgm.*	*divisions per second*	*per cent*	*mgm.*
Liver	0.00303	0	650	0.00150	0	810
Lung	0.00292	− 4	640	0.00150	0	685
Bowel	0.00255	−17*	735	0.00148	− 1.3	745
Skin	0.00281	− 8	635	0.00109	−26*	980
Muscle	0.00292	− 4	670	0.00154	+ 2.7	705
Kidney				0.00146	− 2.7	820

* A large amount of fat in these specimens was hydrolysed to soap. Due to the great viscosity of the solution, pipette drainage was not complete. A small error was also introduced by absorption of radiation by the greater dry weight.

large cup socket to hold the 1.0 ml. brass cups. In this way, the large glass cup and the small brass cup could be used for measurement of radio-iodoprotein, in 1 gram of tissue and 1 ml. of plasma, respectively. Since the larger sliding bar displaced much more air within the chamber, difficulty in keeping a constantly dry atmosphere was encountered. This was corrected by cementing aluminium foil (1.0 mm.) over the opening in the sliding bar sleeve within the electroscope chamber. About 30 per cent of the radiation of iodine was absorbed by this filter. Constant backgrounds were obtained by this method. Fluctuations of 0.000045 to 0.000055 divisions per second in 6 to 10 hours occurred.

The accuracy of tissue analyses for radioactive iodoprotein, using the hydrolytic and evaporation technic, was markedly improved over that for radiobromoprotein, for which the oxidation and silver salt precipitation method was used (1, 2). Tables I and II show the error of the hydrolytic and evaporation technic and may be compared to similar data for bromoprotein in Tables II and III of Paper III (2).

RATE OF DISAPPEARANCE OF RADIOACTIVE PLASMA PROTEIN CONTAINING RADIOACTIVE IODINE FROM THE PLASMA OF NORMAL DOGS

In each experiment described below, the rate of disappearance of the radio-iodo plasma protein from the circulation was observed in a normal morphinized dog. Fairly uniform results were obtained. A composite curve of several such experiments is shown in Figure 1 and may be compared with disappearance curves for radiosulfprotein and radiobromoprotein shown in Figure 1 of Paper IV (1). The greatest rate of disappearance (10 to 25 per cent) occurs during the first hour after injection. The subsequent rate of disappearance is about 2 per cent per hour. In 4 dogs of this group, the average loss of Evans Blue from the circulating plasma during the first hour was 20 per cent.

DETERMINATION OF INTRAVASCULAR PLASMA CONTENT OF TISSUES AND CALCULATION OF PLASMA LEAKAGE

"In addition to curves of disappearance from the circulation of radioiodoprotein, the tissue content of radioactive protein was determined simultaneously. This is necessary because it is not possible to calculate whole plasma loss from blood disappearance curves unless the plasma volume is known with certainty. Since all methods of measuring plasma volume, which depend on dilution of a dye or tagged substance, measure only circulating plasma, reliable calculations of plasma loss are not possible by such techniques under all circumstances, especially during the shock state. The determination of the protein which has actually left the circulation, by analysis of the tissue content of radioactive protein, constitutes a method independent of plasma volume measurements. This method also provides a means of determining whether or not a preferential leakage into certain areas occurs" (5).

In order to calculate the extravascular plasma protein content, the radioactivity due to intravascular radioactive plasma protein was subtracted from the total tissue radioactivity, as measured directly. The intravascular plasma content of tissues (and its radioactivity) was calculated from the hemoglobin content of each tissue and of arterial blood (at the time of death), and the estimated capillary blood hematocrit from equations (*1*), (*2*), and (*3*), derived as follows:

Since

$$\frac{\text{hemoglobin content of tissue blood}}{\text{hemoglobin content of arterial blood}} = \frac{\text{ml. red blood cells per gram tissue}}{\text{ml. red blood cells per ml. blood}}$$

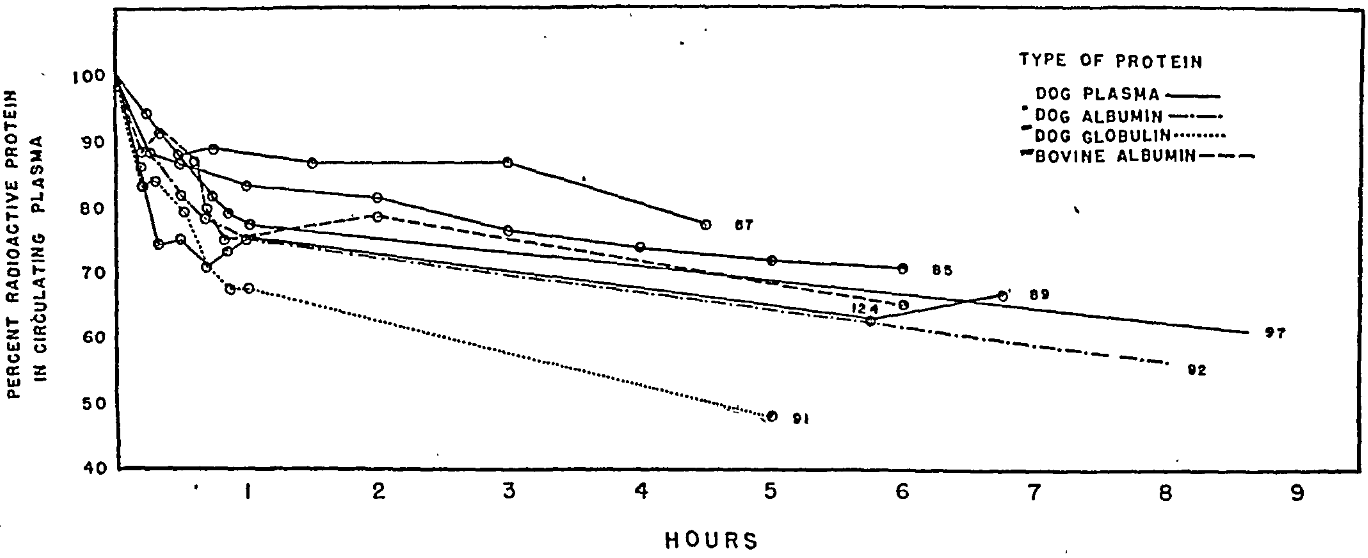

FIG. 1. RATE OF DISAPPEARANCE OF RADIOACTIVE IODOPROTEIN FROM THE CIRCULATING PLASMA OF NORMAL DOGS. DOG NUMBERS ARE GIVEN WITH EACH CURVE.

x Mostly albumin but contained some globulin.

xx Globulin was precipitated by prolonged dialysis and was redissolved with the aid of sodium carbonate.

xxx Prepared from crystallized bovine albumin provided through the courtesy of Prof. E. J. Cohn, Department of Physical Chemistry, Harvard Medical School.

and

$$\frac{\text{ml. red blood cells}}{\text{hematocrit}} = \text{ml. blood}$$

then

$$\frac{\text{hemoglobin content of tissue blood}}{\text{hemoglobin content of arterial blood}} \times \frac{\text{hematocrit arterial blood}}{\text{hematocrit tissue blood}} = \frac{\text{ml. blood}}{\text{gram tissue}}$$

and

$$\frac{\text{ml. blood}}{\text{gram tissue}} \times (1 - \text{hematocrit of tissue blood}) = \frac{\text{ml. plasma}}{\text{gram tissue}}$$

or

$$\frac{\text{hemoglobin content tissue blood}}{\text{hemoglobin content art. blood}} \times \left(\frac{\text{hemat. art. blood}}{\text{hemat. tissue blood}} - \text{hemat. art. blood}\right) = \frac{\text{ml. plasma}}{\text{gram tissue}}$$

$$(1) \quad \frac{\text{ml. plasma}}{\text{gram tissue}} = \text{R}(\text{K} - \text{arterial hematocrit})$$

where

$$(2) \quad \text{K} = \frac{\text{hematocrit of arterial blood}}{\text{hematocrit of tissue blood (capillary)}}$$

and

$$(3) \quad \text{R} = \frac{\text{hemoglobin content of tissue blood}}{\text{hemoglobin content of arterial blood}}$$

Previous studies (8) showed that the average body hematocrit (as determined by radioactive red cells tagged with radioactive iron) was almost never greater than the arterial hematocrit in normal, exsanguinated, or shocked dogs. Simple calculations from these 2 determined hematocrits, and the fact that about 15 per cent of the blood volume is present in the capillary bed, indicated that the ratio (K) $\frac{\text{hematocrit of arterial blood}}{\text{hematocrit of tissue blood}}$ was about 4/3. Therefore the number 1.33 was used for K in applying the formula given above. Undoubtedly K is not a constant from dog to dog or from tissue to tissue in the same dog, but as an approximation, no great error was introduced by the assumption of an arbitrary value for K in the final calculation of leakage. This is shown by the following considerations:

The loss of radioactive plasma from the blood vessels in ml. per gram of tissue was determined by subtracting the extravascular radioactivity per gram tissue of the control dog from that of the shocked dog, divided by the *unit activity* of plasma of the shocked dog. The extravascular radioactivity was determined from the total tissue radioactivity (A) as measured minus the intravascular radioactivity. This latter was determined from equation (*1*) as the intravascular plasma content of the tissue multiplied by the unit radioactivity of circulating plasma at the time of death. The following equation results:

(*4*) Loss in ml. per gram

$$= \frac{[\text{A}_s - \text{R}_s\text{a}_s(\text{K} - \text{H})_s] - [\text{A}_c - \text{R}_c\text{a}_c(\text{K} - \text{H})_c]}{\text{a}_s}$$

where A is total measured radioactivity of 1.0 gram of tissue

$$R = \frac{\text{hemoglobin content of tissue blood}}{\text{hemoglobin content of arterial blood}}$$

a = unit activity of plasma at time of death

$$K = \frac{\text{hematocrit of arterial blood}}{\text{hematocrit of tissue blood}}$$

H = arterial hematocrit

The subscripts c and s refer to control and shocked dogs respectively.

Rearrangement of equation (4) gives

(5) Loss in ml. per gram

$$= \frac{(A_s - A_c) - R_s a_s (K - H)_s + R_c a_c (K - H)_c}{a_s}$$

If the average body hematocrit is never greater than the arterial hematocrit, K will never be less than 1.0. If in equation (5) deviations from the assumed value for K of 1.33 should vary, the error introduced by K varying between 1.0 and 1.5 still can never be great and will tend to cancel out because the hematocrit (usually between 0.2 and 0.8) will not exceed K and therefore not reverse the signs in equation (5). In normal dogs, $(K - H)_c$ is about equal to 1 and in shocked dogs, $(K - H)_s$ could only possibly vary between 0.2 and 1.3 with but a small effect on the final calculations.

As a further precaution to reduce the error from calculations of intravascular blood to a minimum, dogs were exsanguinated at the end of the experiment, and, in isolating tissues for analysis, excess blood and large vessel blood was gently wiped from each piece of tissue. In this way, the intravascular content of blood was reduced to a minimum and as far as possible represented blood in capillaries and very small vessels.

DETERMINATION OF HEMOGLOBIN CONTENT OF TISSUES

Tissues for both radioactivity analysis and hemoglobin determinations were taken from representative areas of each organ and pooled. Skin after shaving was taken from neck, thorax, back, foreleg, and hindleg. Muscle was taken from neck, back, foreleg, and hindleg. Intestine was taken from stomach, small intestine, and colon, after removing the fatty mesenteric border. Lung was taken from several lobes. Damaged legs (tourniquet or burn) were boned and minced in a meat grinder and aliquots taken. The tissues were cut into cubes about 0.5 to 1.0 cm. in diameter and mixed. Four grams were weighed out for radioactivity analysis as described above. Two grams were weighed out for hemoglobin analysis. Muscle and heart were covered with physiological saline and the other tissues with water (40 ml.). The pieces were finely minced with a scissors and allowed to remain overnight in the icebox. In the case of muscle and heart, the residue after centrifugation was extracted with water (9). The suspensions were then filtered through gauze, cleaned with ammonia, diluted with water, and measured in a photoelectric colorimeter (Klett) against standards from arterial blood (taken at the time of death), using 540, 620, and 660 filters. In order to correct for turbidity, the 620 and 660 filter readings were plotted and extrapolated to 540 (9). The value so obtained was subtracted from the reading obtained with the 540 filter and substituted in equation (6) below. Standards were prepared from blood diluted 1000 times and treated in the same way. Since 2.0 grams of tissue were used, the ratio R (equation (3)) is calculated from:

$$(6)\quad \frac{\text{hemoglobin content of tissue per gram}}{\text{hemoglobin content of art. blood per ml.}} = \frac{\text{dilution factor} \times \text{reading for tissue}}{2 \times 1000 \times \text{reading for standard}}$$

Since it was found that the extrapolation method with both filters 660 and 620 gave results but slightly different from those obtained with the 620 filter alone, the latter filter was used in the majority of the analyses. The reading as obtained was subtracted from that found with the 540 filter.

Although inaccuracies in the hemoglobin measurements are unavoidable, due to methemoglobin formation, turbidity, incomplete extraction of tissue, and possibly other factors, the red cell volume in ml. per gram of tissue by this method agreed sufficiently well with simultaneous red cell volume determinations made by the radioiron red cell technic[7] to permit acceptance of the hemoglobin method as a good enough approximation to the facts (cf. Table VI of previous publication (5)).

RESULTS

A. Hemorrhagic shock

In experiments with radiobromoprotein (1), the plasma loss in hemorrhagic shock was found not to exceed that of the normal dog. In the following experiments, plasma loss into tissues of the dog in irreversible shock (*i.e.*, not lastingly responsive to a transfusion of all blood removed) is compared with that of a dog in reversible shock (*i.e.*, responsive to a transfusion of all blood removed).

Experiment No. I-7: Two dogs (Nos. 98 and 99) were bled into shock by a hemorrhage of 4.1 per cent and 2.7 per cent of the body weight, respectively. After 7.5 hours in shock, when the blood pressure was 20 mm. Hg, Dog No. 98 was given a transfusion of dog plasma (4.5 per cent of the body weight) containing radioiodoprotein. At the same time, a transfusion of dog plasma (3.7 per cent of the body weight)

[7] In collaboration with Gibson and Peacock (8).

containing radioiodoprotein was given to Dog No. 99, which had been in shock for 3 hours, when the blood pressure was 30 mm. Hg. The same quantity of radioiodoprotein was given to each dog in proportion to the body weight. Dog No. 98 did not recover, and when the blood pressure again declined to 25 mm. Hg, the blood pressure of Dog No. 99, which was recovering, was 100 mm. Hg. Both dogs were exsanguinated 3 hours after their transfusion. Dog No. 98 yielding a volume of blood which was 0.67 per cent and Dog. No. 99, 2.5 per cent of the body weight. Tissue samples for analysis were taken immediately from both, with results shown in Table III, in which the ratio of radioactivity content per gram of tissue of Dog. No. 98 (irreversible shock) to that of Dog No. 99 (reversible shock) is given, as well as the plasma loss in ml. per whole organ, calculated from the difference in extravascular radioiodoprotein content per gram of tissue between Dogs Nos. 98 and 99; *i.e.*, the excess of radioiodoprotein in the tissues of Dog No. 98.

TABLE III

*Ratio of radioactivity (radioiodoprotein) of tissues of dog in irreversible hemorrhagic shock as compared to control dog in reversible hemorrhagic shock, including plasma loss in whole organs in shocked (irreversible) as compared to control (reversible shock) dog**

Experiment I–7

Tissue	Ratio *per cent*	Plasma loss *ml.*
Liver	13	0
Lung	32	0
Kidney	87	0
Intestine	68	0
Spleen	112	1
Skin	41	0
Heart	62	0
Muscle	121	9.5
Total		10.5

* When radioiodoprotein is used, any ratio below 95 or above 105, except in muscle, is taken to represent a difference outside of experimental error. This error is much smaller than in the case of radiobromoprotein (1, 2). The significance of such ratios is not evident in the ratio figures themselves since two factors of importance affect the calculation. These are the relative weights of the organ and the relative radioactivity measurements of such organs per gram. For example, if a ratio of 200, derived from a radioactivity of 0.001 div. per sec. on the electroscope in the control and a radioactivity of 0.002 div. per sec. in the shocked dog, applies to a gram of tissue of a given organ (A); and the same ratio derived from a radioactivity of 0.0001 div. per sec. in the control and 0.0002 div. per sec. in the shocked dog, applies to a gram of tissue of another organ (B) of the same weight, the difference in radioactivity per gram of tissue will be 0.001 for organ A and 0.0001 for organ B. The total radioactive protein content of organ A will be ten times that of organ B and the quantitative loss involved is obviously far less in organ B, even though the ratio is the same for both. The relative activities of various tissues in normal dogs are shown in another publication (1). The quantitative significance of an increased ratio is of little consequence in an organ like the kidney, which is small in weight, and the same is true when a large organ has a very low order of radioactivity.

Since the unit activity of circulating plasma found at the end of the experiment was used in calculating the volume of plasma loss, the values for loss obtained represent the greatest possible loss and are probably high.

Except for muscle and spleen, all the tissues of Dog No. 98 contained less extravascular plasma than those of Dog No. 99. There is, accordingly, no evidence of greater leakage of plasma in the dog in irreversible hemorrhagic shock as compared to the dog in reversible shock. On the contrary, the former, probably because of less efficient capillary circulation, seems to have displayed a slower rate of escape of iodoprotein from the circulation.

Since no significant leakage of plasma was noted following a plasma transfusion in irreversible hemorrhagic shock, it was considered desirable to see whether plasma leakage was significantly increased by a subsequent saline infusion, which is widely held to be responsible for washing protein out of the circulation.

Experiment No. I–9. Two dogs (Nos. 122 and 123) were bled into shock by a hemorrhage of 3.8 per cent and 4.0 per cent of the body weight, respectively. After 5 hours in shock, when its blood pressure was 30 mm. Hg, Dog No. 122 was given a transfusion of all shed blood, containing 20 ml. radioiodoalbumin (bovine) with a pressor response to 90 mm. Hg. Four hours later the blood pressure was 45 mm. Hg. Since the dog was in "irreversible" shock, a saline infusion of 1500 ml. was given. This resulted in a maximum rise in blood pressure to 95 mm. Hg, with a subsequent decline to 60 mm. Hg, three and one-half hours later when the dog was exsanguinated (500 ml.). All tissues were edematous and there were slight hemorrhages in the jejunum and colon. Radioactive red cells were injected (8) and the tissues were analyzed for blood content on the basis of radioactive iron content. These data will be published elsewhere.

After 2 hours in shock, with a blood pressure of 65 mm. Hg, Dog No. 123 received all shed

blood containing 26 ml. of radioiodoalbumin (bovine). Four hours later, the blood pressure was 100 mm. Hg. This dog was therefore reversible. A saline infusion of 2000 ml. was then given. The pressure rose to 140 mm. Hg and three and one-half hours later was 90 mm. Hg when the dog was exsanguinated (600 ml.). All tissues were edematous and there were slight hemorrhages in the jejunum.

As a further control for comparison, a normal dog (Dog No. 124) was given 24 ml. of radioiodoalbumin (bovine). Four hours later, 1500 ml. saline were given intravenously and three and one-half hours later the dog was exsanguinated (1150 ml.).

Radioiodoalbumin was given in proportion to body weight in all but this control dog. Correction for this was made in the final calculations. The hemorrhagic jejunum of Dog No. 123 was analyzed separately for plasma content per gram and found to be about the same as for the rest of the intestine in terms of total radioactivity and less on the basis of extravascular radioactivity.

If the calculated final total plasma volumes of these 3 dogs (all of which received equivalent saline infusions) is taken to equal 10 per cent of their respective body weights minus the blood removed plus the volumes of blood and saline infusions, the tissue radioactivity data show that the loss in plasma volume of the irreversibly shocked dog was 10 per cent greater than in the normal dog and the loss of the reversibly shocked dog 3 per cent greater than in the normal dog. One must conclude, therefore, that saline infusion increases the leakage of plasma protein in shock and more markedly in irreversible shock. The tissues showing most significant leakage were muscle, intestine, lung, and liver (Table IV). However, it is unlikely that the loss of 10 per cent of the plasma volume after the large saline infusion in the irreversibly shocked dog was responsible for the subsequent decline in blood pressure, especially since the blood pressure had previously declined to 45 mm. Hg following whole blood tranfusion.

"Since the forces operating to produce lethal shock are not necessarily the same in all types of shock, it became necessary to obtain data on the question of generalized increase in capillary permeability in conditions other than hemorrhage. Tourniquet shock and burn shock differ from hemorrhagic shock in that (*1*) a local loss into areas of injury occurs and (*2*) such injured tissues perhaps may liberate a substance capable of affecting capillary permeability in general" (5).

TABLE IV

Plasma loss in whole organs in shock dogs (irreversible and reversible) as compared to normal control dog following transfusion and saline infusion

Experiment no. I–9

Tissue	Plasma loss: Irreversible, Dog no. 122	Plasma loss: Reversible, Dog. no. 123
	ml.	*ml.*
Liver	23	49
Lung	33	2
Kidney	10	0
Intestine	27	41
Spleen	3	0
Skin	4	4
Heart	0	0
Muscle	140	0
Total	240	96

B. Tourniquet shock.

A tourniquet was applied at the groin to one or both legs of a dog under morphine (3 mgm. per kgm.) and left on for 5 to 10 hours. Plasma volume was then measured and radioiodoprotein was given to this dog and to a normal morphinized dog in equivalent amounts. The tourniquet was removed and the same measurements as in Experiment I–7 made. Protocol data are given in Table V. Table VI lists the results of tissue analyses of 4 dogs (I–2A, I–2B, I–3, and I–4) showing plasma protein loss in various tissues including the tourniqueted extremities. While the results indicate loss of protein into some tissues other than the extremities, such loss involves only 1 to 3 tissues and in no case is the total loss outside of the extremities quantitatively significant, from the point of view of plasma protein depletion. There is, therefore, no evidence that a change in permeability to proteins, outside the area of injury, if it exists, is of consequence in the development and progression of shock following the removal of a tourniquet.

The volume of plasma lost into the tourniqueted extremities is of course large. The gain in weight was not determinable in Experiments I–2A and I–2B, where both legs were injured, but in Experiments I–3 and I–4 the weight increase (above the opposite normal leg) was much

greater than the plasma gain determined by radioactivity assay of the tissues (Table V). The discrepancy is far in excess of errors in method. The difference, therefore, in major part, represents a gain in tissue water drawn from outside areas.

The plasma gain in the injured extremities in 3 of the 4 shocked dogs listed in Table VI is significantly less than the plasma loss found by circulating plasma volume determinations (Table V). It was pointed out elsewhere (1) that in deep shock, plasma volume measurements by the dye technic may overestimate loss because the segregation of more plasma into the stagnant peripheral bed as shock deepens (a progressive rise in the amount of trapped plasma) will increase the extent of incomplete mixing and so give too low a dye dilution figure in terms of the total plasma volume. Hence one may expect a discrepancy between the dye method and the tissue radioactivity method, the former showing a falsely higher loss, the more stagnant the peripheral bed becomes. But the much greater total fluid loss into the leg, found by actual weight change, than is disclosed by the dye method again points to a large access of tissue water derived from extravascular sources.

C. Burn shock

Three experiments were performed in which one or more extremities were immersed under ether anesthesia for varying intervals in water at 98° C. (Tables V and VI). In Experiments I–5 and I–6, radio-iodoprotein was injected intravenously into a normal dog and into the burned dog before the burn was produced. In Experiment I–8, the technic was varied in order to see whether the capillaries were more permeable to protein in a severely burned than in a mildly burned animal. For this purpose, 2 dogs were burned, one for 120 seconds, the other for 20 seconds. Four hours before death of the

TABLE V

Protocol data in tourniquet shock and burn shock and comparison of plasma loss into injured extremity by three criteria

Type of shock	Exper. no.	Dog no.	Dog wt.	$\frac{Ws}{Wc} \times \frac{Ic^*}{Is}$	Duration of tourniquet (hours) or burn (seconds)	Duration of exper. after removal of tourniquet or burn	Blood pressure at end of exper.	Volume of exsanguination at end of exper.	Hematocrit increase	Decrease in P.V. by dye method†	Wt. increase of injured extremity	Plasma loss into injured extremities by radioactivity analysis of tissue‡	Remarks
			kgm.			*hours*	*mm. Hg*	*ml.*	*per cent*	*ml.*	*grams*	*ml.*	
Tourniquet	I–2A	84	8.7	1.0	5	5.7	75	270	8	300		102	Tourniquet on both hindlegs.
		85	6.9		control		110	160	0				
	I–2B	86	10.6	0.93	8	4.0	0	0	13	307		132	Tourniquet on both hindlegs.
		87	5.1		control		110	200	0				
	I–3	88	11.8	0.92	10.5	5.7	10	30	16	257	360	122	
		89	14.1		control		120	500	0				
	I–4	90	13.4	1.0	7.5	11.5	30	100	4	220	435	180	After 7 hours the increase in hematocrit was 13 per cent (Dog 90).
		92	8.2		control		105	150	0				
	I–5	94	8.5	0.98	45	27	0	0	1	45	121	190	Burn of foreleg and hindleg. B.P. remained at 80 mm. Hg and dropped to 30 mm. Hg after a small dose of nembutal (Dog 94).
		95	5.0		control		110	200	0				
Burn	I–6	96	11.6	1.0	90	18.5	0	0	16	230		445	Burn of both hindlegs.
		97	10.6		control		110	480	2				
	I–8	200	11.8	1.0	120	22	30	0	8	785	69	24§	Plasma infusion (325 ml.) with radioprotein.
		201	10.1		20	21	95	150	11	250	171	38§	Plasma infusion (270 ml.) with radioprotein.

* Formula showing relative proportion of radioprotein dosage to weigh of shocked versus control dog, when W = wt. of dog, I = volume of radioactive plasma injected, c = control dog and s = shocked dog.

† In burn experiments, plasma volumes by dye method were complicated by marked hemolysis.

‡ Data from Table VI.

§ Since tagged infusion was given four and one-half hours before end of experiment, loss during this period only was determined.

more severely burned dog, a plasma infusion containing radio-iodoprotein was given simultaneously to both dogs.

The extravascular radioactive protein content of the tissues in all 3 experiments showed no significant loss in the plasma protein content of the unburned tissues of the burned dog in comparison with the same tissues in the unburned control dog (Experiments I–5 and I–6) or the less severely burned control dog (Experiment I–8).

In Experiment I–5 and I–6, there is no correspondence between the plasma volume loss into the burned extremity, as determined from radioactive plasma protein content, and the loss as measured by intravenous dye. The unavoidable hemolysis of blood in burned dogs affects the reliability of the latter method.

In Experiment I–8, the radioactive protein content was injected too late to permit a determination of total plasma protein loss into the burned area. In the severely burned dog, the very small increase in weight of the burned extremity suggests that peripheral vascular collapse was due to factors other than plasma volume deficiency.

Although the extent of the plasma protein loss into the tissues in the foregoing experiments cannot be assessed with complete accuracy (±10 to 15 per cent), the loss outside of areas of injury involves only 1 or 2 tissues, which vary from one experiment to another, and, in any case, is not quantitatively significant.

The calculation of plasma volume loss into tissue is based on the assumption that if such loss occurs outside of areas of local injury, whole plasma is lost. If greater capillary permeability to the non-protein than to the protein fraction of the plasma should exist, the disproportion between the loss of the protein and the non-protein fraction of plasma would be reflected in a shift of the total protein concentration. Since the latter does not increase in hemorrhagic shock (5), plasma if lost would be in proportion to protein loss in hemorrhagic shock, except perhaps when saline infusions are given in the late shock phase. The rise in total protein concentration in burn shock and in tourniquet shock may be explained as due to a disproportionate loss of the non-protein fraction into or outside the injured area. Such loss, if substantial, into tissues outside the injured area, would not be likely without a loss of a measurable amount of protein. Since no protein is lost (*i.e.*, no loss of consequence, except in a sporadic and inconsistent manner involving 1 or 2 tissues varying from one experiment to another) into areas outside the locally traumatized area in either burn shock or tourniquet shock, our assumption that no plasma is lost outside of areas of local injury seems justified. Evidence indicates that water is drawn from outside areas and lost into the injured areas, *i.e.*, uninjured areas are dehydrated.

TABLE VI

Plasma loss in whole organs of dogs in tourniquet or burn shock as compared to control dogs

Plasma loss							
Exper. no.	TOURNIQUET SHOCK				BURN SHOCK		
	I–2A	I–2B	I–3	I–4	I–5	I–6	I–8*
	ml.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
Liver	0	0	2.6	0	0	0	15
Lung	0	0	7	2	0	0	9
Kidney	1.3	0	0.7	0	2.1	0	0
Intestine	33	0	0	0	20	0	0
Spleen	2	0	0.4	1.4	0.7	0	0
Skin	3.6	3.5	0	0	0	5	3
Heart	0	0.4	0	0	0	0	0
Muscle	0	43	7	3.5	0	7	10
Injured extremities	102	132	122	180	190‡	445†	24‡
Totals	143	179	140	184	213	457	61

* The control dog in this experiment was also burned, one more severely than the other to compare severe and mild burn shock states.

† If loss of plasma is assumed to have occurred early after the burn, these values would be halved.

‡ Plasma loss into the burned legs was calculated by comparison with values for intact muscle and skin for both dogs. Plasma loss in milder burn was 38 ml. Small loss was due to the fact that leakage occurred before radio-iodoprotein was given (four and one-half hours before the end of the experiment).

COMMENT

The foregoing evidence does not support the idea of capillary permeability causing leakage of plasma from the blood to the tissues except in localized areas of injury. The widespread belief that the capillaries throughout the body "leak" plasma is presumably based on the common observation in man and animals, dead of shock, that the tissues are wet or hemorrhagic. While saline infusions in particular will facilitate an increased rate of escape of plasma protein, further evidence is provided in another publication (10) that *such leakage is the result of therapy* and is not seen in untreated shock, except in the case

of intestinal bleeding in burns. But even in burns, it remains to be shown that hemorrhage into the intestine or elsewhere is of a sufficient order of magnitude to contribute seriously to the hemodynamic deficiency caused by the local plasma loss in the burned area. A change in permeability of the general capillary bed, whether it exists or not, cannot be said to bear any relation to the shock syndrome. Saline therapy causes some loss of plasma protein into tissues. To what extent the shock state is thereby altered cannot be assessed.

The evidence is clear also that when the deficient plasma volume due to loss into an area of injury is corrected, circulatory collapse is not due to a progressive decline in plasma volume from leakage of plasma outside of the area of injury. While a decline in effective circulating volume may occur during this subsequent phase of circulatory collapse, it is due in part to continued leakage into the injured area and in part to progressive peripheral stagnation. In irreversible hemorrhagic shock, death ensues even when blood or plasma is given so as to produce a plethora. Since leakage of plasma from the generalized capillary bed does not occur, it is not the cause of death.

CONCLUSIONS

1. Plasma proteins tagged with radioactive iodine were used to study the capillary leakage hypothesis in hemorrhagic, tourniquet, and burn shock. No evidence of leakage due to a change in the permeability of the generalized capillary bed was found. Tagged plasma proteins escaped into areas of injury in considerable amounts, but not into untraumatized areas. This was also true after plasma infusion.

2. There is also evidence to show that the general capillary bed does not become more permeable to plasma proteins or plasma in the late or irreversible phase of hemorrhagic shock following transfusion.

3. Following saline therapy in hemorrhagic shock, plasma proteins are carried out of the blood stream with saline. This occurs to a greater extent in irreversible than reversible hemorrhagic shock. The volume of dilute plasma lost in this way is small.

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TRAUMATIC SHOCK. VIII. STUDIES IN THE THERAPY AND HEMODYNAMICS OF TOURNIQUET SHOCK[1]

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Although traumatic shock created by the release of a tourniquet applied for some hours to an extremity is an unusual clinical condition, it is comparable to other clinical states such as burns, intestinal obstruction, certain high velocity projectile wounds (1), etc., in which a large and more or less rapid loss of plasma from the circulation is the most obvious shock-inducing agent. While special features of these various conditions obscure the effects of the mere loss of plasma (*e.g.*, in tourniquet and high velocity wounds, muscle is crushed; in burns, tissue is burned; and in simple intestinal obstruction, electrolytes and water are lost by vomiting), tourniquet shock constitutes a more controllable and possibly less complicated type than others for an experimental study of shock resulting primarily from plasma loss.

Numerous studies (2 to 7) of tourniquet shock are almost uniformly agreed that death usually occurs regardless of the therapy employed, unless the tourniqueted extremities are refrigerated (5, 6) immediately upon release of the tourniquets or tightly taped, so as to prevent the escape of plasma from the circulation (7). That taping is sufficient to prevent the onset of shock would argue against any relationship between the onset of shock and the muscle damage inflicted by the tourniquet at its site of application. It therefore is not surprising to note the failure to date of efforts to isolate an etiologically significant "toxin" from the crushed muscle.[2] In these circumstances, it is all the more perplexing to find in the reports published to date almost uniform failure to achieve a cure of the shock state by early and adequate replacement of the plasma lost into the injured extremities.

A revaluation of the experimental set-up and the effectiveness of replacement therapy was therefore regarded as necessary. This report will present evidence that, if the proper circumstances are created, adequate replacement therapy is successful in an advanced state of shock. Data will also be given on the response to succinic acid therapy. Certain differences in response to blood volume replacement between hemorrhagic and tourniquet shock will be discussed.

METHOD

A review of the technics hitherto employed reveals that all experiments so far reported were carried out under anesthesia—usually a barbiturate. The notorious effect of anesthetics on the shock state led us to omit all anesthetics or sedatives except intravenous or intramuscular morphine sulphate (3 to 6 mgm. per kgm.) which was given

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[2] The presence of *Cl. welchii* in dog muscle has long been known. Aub *et al.* (A Toxic Factor in Experimental Traumatic Shock—New England J. Med., 1944, 231, 71) have frequently found this organism in large numbers in the fluid transudate from damaged muscle in the dog. Large quantities of such fluid were found to be toxic and capable of producing shock. Filtrates from cultures of *Cl. welchii* isolated from muscle were also capable of inducing shock.

Such evidence does not establish a case for the etiological significance of this organism in tourniquet shock, for the following reasons: (*1*) Shock due to Welch toxin cannot be cured by plasma or albumin therapy. Tourniquet shock can be cured by these agents; (*2*) The death from tourniquet shock is extraordinarily rapid, a matter of a few hours usually, following release of the tourniquet, so that too little time is available for the development of enough toxin to be the lethal agent; (*3*) The amount of fluid transudate required by Aub *et al.* to induce fatal shock was usually far in excess of that produced by a single animal; (*4*) That Welch toxin is lethal is well known, but the type of shock produced, particularly the severe hemolytic effect of Welch toxin, is not seen in tourniquet shock; (*5*) Aub *et al.* found no correlation between the number of organisms cultured from damaged muscle and the severity of shock; some of these organisms in fact did not produce potent toxin; (*6*) Alteration of the course of tourniquet shock by pretreatment with antitoxin has not been demonstrated.

TABLE I

Therapy of tourniquet shock

Experimental groups	Group result	Succinic acid survival	Dog no.	Dog wt.	Tourniquet on	Before treatment: Time*	Before treatment: BP	Before treatment: Art. hem.	Before treatment: P.V.	Before treatment: R.C.V.‡	Therapy: Time*	Therapy: BP	Therapy: Art. hem.	Therapy: 25 per cent bov. alb.		Therapy: 5 per cent bov. alb.		Therapy: Saline		Therapy: Fluid by stomach	
																					Kind
				kgm.	hours	hr. min.	mm. Hg		ml.	ml.	hr. min.	mm. Hg		ml.	ml. per kgm.	ml.	ml. per kgm.	ml.	ml. per kgm.	ml.	
A. Physiol. saline intravenously (5 hour tourniquet application)	Survived 5 of 6		S-68	10	5						7	70	74					1150	115		
			S-69	11.4	5						5 10	75	71					2100	184		
			S-70	15.5	5						8 25	100	48					750	49		
			S-71	9.6	5						8 25	115	67					750	78		
			S-72	19	5						3 45	45	70					3240	170		
			S-73	12.3	5						7 5	65	69					1000	81		
B. Physiol. saline intravenously (10 hour tourniquet application)	Died 6 of 6	0 of 6	S-51	9.5	10						2 10	40						1100	116		
			S-52	10.0	10						2 43	40	70					1300	130		
			S-53	11.3	10						2 50	65	58					1550	137		
			S-54	14.0	10						2 5	40						1600	114		
			S-55	12.2	10						2 45	60	58					1350	110		
			S-56	10.9	10						2 40	60	60					1600	147		
C. 5 per cent sodium chloride intravenously (20 ml. per kgm) + water in stomach	Died 5 of 5	0 of 4	S-62	5.5	10						1 30	60	64					110	20•	500	Water
			S-63	5.5	10						2	60	67					110	20•	1000	Water
			S-64	8.0	10						5	70	75					160	20•	1200	Water
			S-65	5.5	10						1 30	55	81					110	20•	650	Water
			S-67	5.9	10						3 30	60	90					120	20•	1200	Water
D. 5 per cent bovine albumin (40 to 50 ml. per kgm.)	Survived 5 of 7	2 of 4	B-2	12.7	8¾						9 40	60				600	47				
			B-3	5.9	8¾						4 20	40				250	42	200	34		
			B-11	10.0	8						6 15	40	73			400	40				
			B-12	11.3	8						13	60	70			500	44				
			B-13	18.2	8	8 30	100	69	615	760	10	60				870	48				
			B-14	13.6	8						6	70	70			565	42				
			B-15	8.2	8						3 35	50				330	40				
E. 5 per cent bovine albumin (unlimited)	Survived 6 of 7		B-16	14.5	9½						6 30	60	63			800	55				
			B-17	12.7	11	3 30	90	71	370	505	4 11	30				2275	179				
			B-19	13.2	10¼	6 30	100	67	440	550	12 10	50	65			1000	76				
			B-21	10.0	10	6	60	82	220	485	6 42	60				1200	120				
			B-22	10.9	10	5 15	50	71	180	280	5 45	50				900	83				
			B-24	11.0	9						5 10	60				1000	91				
			B-28	10.0	10						4	50				1500	150				

* Time from removal of tourniquets.

† Sodium chloride (5 grams per ml.) added to albumin.

‡ Red cell volume determined with radioactive red cells by Gibson and Evans.

× Deviation of the plasma volume found after treatment, from the expected volume calculated on the basis of the intravenously administered fluid—expressed as a percentage of the plasma volume measured in shock.

• 5 per cent sodium chloride solution.

§ First noted time, blood pressure, and hematocrit, follow the 5 per cent saline therapy, and the second set of recordings refer to succinic acid therapy (section C).

before and once or twice during the application of the tourniquets. The extremities became insensitive after some time and no further drug was necessary (a few exceptional instances required a single supplementary dose of 16 mgm. intramuscularly), so that the experiment thereafter was carried through with no further medication except therapeutic substances as noted below.

Following hairclipping, washing, and the application of

TABLE 1—*Continued*

Therapy of tourniquet shock

Therapy			Best response		After treatment									Result	Pathological findings and remarks
Succinic acid		Succinic acid	Time*	BP	Time*	BP	Art. hem.	Plasma volume			Red cell volume‡				
	Saline	When given						Calc.	Found	Net²	Calc.	Found	Net		
mgm. per kgm.	*ml.*		*hr. min.*	*mm. Hg*	*hr. min.*	*mm. Hg*		*ml.*	*ml.*	*per cent*	*ml.*	*ml.*	*ml*		
			11	90		90								Survived	
			6 5	95	10 50	90	52							Survived	
			10 50	110										Survived	Probably not in shock.
			11	120										Survived	Probably not in shock.
			4 15	115	10 35	80	53							Died—22 hours	Pulmonary congestion and edema. No hemorrhage in gut.
			7 30	105	11	100	48							Survived	
89		Continuously	3	90	8 15	55	52							Died—8 hours 35 minutes	Wet tissues. Empty bladder. No hemorrhage in gut.
210		Continuously	3 30	100	9 30	70	49							Died—15 hours	Pulmonary congestion and edema. No blood in gut. Sl. muc. hem.
251		Continuously	4 45	90	8 30	35	47							Died—8 hours 45 minutes	Pul. congestion (1 lobe). Empty bladder. No hemorrhage in gut.
82		Continuously	2 15	120	5 15	40	58							Died—5 hours 25 minutes	Wet tissues. Basal pul. edema. Empty bladder. No hemorrhage.
78		Continuously	4 45	110	8 30	55	62							Died—9 hours	Wet tissues. Pul. congestion. Empty bladder. Blood in duodenum and jejunum.
275		Continuously	6 20	110	8 25	40	52							Died—8 hours 45 minutes	Wet tissues. Sl. pul. congestion. Empty bladder. No gut hemorrhages.
			1 40§	90	2 40	20	53								
730	400	3 hours at BP 20	3 15	90	4 40	35	49							Died—5 hours	Pul. congestion and edema. Empty bladder. Blood in duodenum.
			2 30	75	4 00	30	66								
730	400	4 hours at BP 30	4 20	70	5 25	30	61							Died—6 hours 10 minutes	Pul. congestion. Empty bladder. Hemorrhagic duodenum.
			5 25	85	8 30	75	69								
														Died—24 hours	Stomach and gut empty. No hemorrhage.
			1 45	75	2 40	20	75							Died—4 hours 30 minutes	Empty bladder and gut. Bloody bowel. Pul. congestion.
730	400	2 hours 40 minutes at BP 20	3	65	3 35	15	56								
			3 40	110	4 50	35	81								
850	500	4 hours 50 minutes at BP 35	5 30	120	6	40	68							Died—6 hours 15 minutes	Empty bladder. Large hemorrhage in large and small bowel.
			10 20	135	12 40	110								Survived	
127		9 hours 15 minutes at BP 50	5	90	11 30	70								Survived	
40		8 hours 15 minutes at BP 80	6 35	110	12	90	54							Died—20 hours	
70		with albumin	13 15	110	13 50	95	39							Died—28 hours	Pul. congestion and atelectasis. Congested liver.
			11 30	115	11 30	115	46	1485	1190	−48	760	820	+60	Survived	
			6 30	105	12 15	80	40							Survived	
200		with albumin	7 35	120	12 30	80	51							Survived	
			6 45	115	11 50	110	44							Survived	
			4 35	90	13 45	85	30	2645	1030	−436	505	500	−5	Died—16 hours	Cl. Welchii in leg muscles.
			14	100	14	100	32	1440	1130	−70	550	540	−10	Survived	
			7 15	120	10 35	110	33	1420	415	−456	485	465	−20	Survived	
			5 55	130	9 40	95	35	1080	410	−372	760	345	+85	Survived 32 hours	Purulent infection of legs (streptococcal).
			5 30	95	10	85	44							Survived	Omentum exteriorized for microscopy.
			5	105	15	105								Survived	Omentum exteriorized for microscopy.

an antiseptic (tincture of iodine or zephiran), 5 or 6 turns of heavy-walled rubber tubing at maximal manual tension were applied to both legs as high on the thigh as possible and allowed to remain for 5 hours, the interval usually employed by previous investigators. Upon release, the extremities swelled, but shock was either slow to appear, *i.e.*, frequently not for many hours (5 to 7), or it did not occur at all. Furthermore, the shock state so induced was effectively treated by saline solution alone. This was so contrary to all previous experience that the difference was regarded as due wholly to the omission of barbiturates.

To induce rapid and severe shock, we altered the technic by merely prolonging the time of tourniquet application to 8 to 11 hours. Nearly all such dogs (79 out of 80) developed profound shock. If no treatment was given, death resulted usually in less than 6 hours and always in less than 12 hours.

Autopsy showed extensive muscle damage, with degeneration of muscle fibers and some hemorrhagic extravasation into the crushed muscle. The swelling distal to the

TABLE I—*Continued*

Experimental groups	Group result	Succinic acid survival	Dog no.	Dog wt.	Tourniquet on	Before treatment: Time*	Before treatment: BP	Before treatment: Art. hem.	Before treatment: P.V.	Before treatment: R.C.V.‡	Therapy: Time*	Therapy: BP	Therapy: Art. hem.	Therapy: 25 per cent bov. alb.		Therapy: 5 per cent bov. alb.		Therapy: Saline		Therapy: Fluid by stomach	
																					Kind
				kgm.	*hours*	*hr. min.*	*mm. Hg*		*ml.*	*ml.*	*hr. min.*	*mm. Hg*		*ml.*	*ml. per kgm.*	*ml.*	*ml. per kgm.*	*ml.*	*ml. per kgm.*	*ml.*	
F. 25 per cent bovine albumin (10 ml. per kgm.)	Died 5 of 5	0 of 3	B-5	8.2	8¾						7	40		80	10						
			B-6	7.8	8¾						7 45	50		80	10						
			B-7	10	8¾						6 40	50		100	10						
			B-8	11.4	8¾						10 45	40	60	110	10						
			B-9	9.1	8¾						8 15	60		91	10						
G. 25 per cent bovine albumin (10 ml. per kgm.) + saline in stomach	Survived 1 of 2		B-25	11.8	11½	4	100	55	385	306	5	60		120	10					1300	Saline
			B-26	10.5	11½						4 10	55		105	10					1900	Saline
H. 25 per cent bovine albumin (20 ml. per kgm.)	Died 5 of 5		B-38	12.3	10	2	90	77	240	480	3 40	60	77	240	20						
			B-39	14.1	10						2 20	65	74	280	20						
			B-40	11.4	10						2 30	60	80	230	20						
			B-41	9.2	10	2	105	78	136	293	3	55	80	180	20						
			B-42	11.8	10						3 10	65	77	230	20						
I. 25 per cent bovine albumin (20 ml. per kgm.) + saline in stomach	Survived 5 of 6		B-31	13.2	10	2	95	67	342	430	4 30	60		264	20					1250	Saline
			B-32	11.4	10						2	35	60	224	20					2600	Saline
			B-33	7.7	10	2 20	95	65	220	221	3 30	70		153	20					1250	Saline
			B-34	9.2	10						2 30	65	62	180	20					3000	Saline
			B-35	13.2	10						5 10	65	69	264	20					1250	Saline
			B-36	10.0	10						3 20	65	74	200	20					1250	Saline
J. 25 per cent bovine albumin (20 ml. per kgm.) + water in stomach	Survived 3 of 6		B-43	7.7	9¾						2 10	55	67	155†	20					1500	Water
			B-44	6.8	9¾						3 30	50	81	136†	20					750	Water
			B-45	13.2	9¾	2 40	100	71	290	620	6 10	60		265†	20					500	Water
			B-46	9.2	9¾						3 50	65	74	180†	20					1950	Water
			B-47	26.4	9¾	2	90	62	730	1060	7 30	75	67	500†	20					1650	Water
			B-48	18.2	9¾	3 13	90	69	455	702	4 20	65		364†	20					2000	Water
K. Dog plasma (40 to 50 ml. per kgm.)	Survived 2 of 2		4	14	9¼						8 50	70				(Plasma)	44				
			6	14.5	11½						7	70	70			(Plasma)	41				

site of tourniquet application was pronounced, but it did not occur until after removal of the tourniquets. The arteries and veins were patent and there was no evidence of mechanical interference with blood flow during the shock state.

Following release of the tourniquets, no incision or other manipulation of the lower extremities was made, for it was evident early in our experience that such extremities were readily infected thereby. Accordingly, blood sampling and intravenous therapy were done under local anesthesia in the neck in most of the experiments reported below. The data gathered included determinations of arterial and venous (right heart) oxygen concentration, oxygen consumption (tracheal cannula inserted under local anesthesia), cardiac output (8), arterial hematocrit and arterial blood pressure (carotid artery), before shock and before and after therapy during shock. In some experiments, plasma and red cell volumes were measured,[3] using the dye technic and red cells tagged with radioactive iron.

The experiments were usually carried out by studying a given type of therapy in 5 or 6 dogs simultaneously, thus eliminating variations in response to tourniquet application, which seem to depend on environmental conditions. (Thus we have the impression that dogs go into very rapid and severe shock more readily on hot humid days.)

After release of the tourniquets, the dogs became quiet and apathetic, the legs swelled rapidly, and shock was obviously present before the blood pressure began to fall, as indicated by the dull apathetic state of the animal and an already markedly lowered cardiac output (8). In the 61 dogs of this report, therapy was started only when the blood pressure had fallen to 70 mm. Hg or lower. While this level of blood pressure is regarded as "the critical level" in hemorrhagic shock, a higher level is critical for tourniquet shock. Hence, the treatment may be regarded as having been applied after poor blood flow had been present for some time.

The therapy given was intended only for cure of the shock state. Successful therapy is herewith defined as recovery from shock and survival for a minimum of 24 hours after removal of the tourniquets, regardless of whether or not the dog subsequently died or was sacrificed because of massive necrosis or sepsis. It is possible that

[3] These data, gathered, in collaboration with Gibson and Evans, for the purpose of determining peripheral vascular stagnation, will appear elsewhere.

TABLE I—*Continued*

Therapy			Best response		After treatment									Result	Pathological findings and remarks
		Succinic acid	Time*	BP	Time*	BP	Art. hem.	Plasma volume			Red cell volume‡				
	Saline	When given						Calc.	Found	Net×	Calc.	Found	Net		
mgm per kgm	*ml.*		*hr. min.*	*mm. Hg*	*hr. min.*	*mm. Hg*		*ml.*	*ml.*	*per cent*	*ml.*	*ml.*	*ml.*		
100	200	after albumin BP 40	7 15	95										Died—13 hours 20 minutes	Small amount of blood in jejunum.
100	200	after albumin BP 40	8 40	100										Died—15 hours 45 minutes	No blood in gut.
80	100	after albumin BP 40	7 10	90										Died—13 hours 45 minutes	No blood in gut.
			14	90	18 45	85								Died—28 hours	
			10 15	90	15 15	80								Died—24 hours	(One tourniquet off too early.) Omentum exteriorized.
			5 40	100	8 30	95	34 •	505	950	+115	306	332	+26	Survived	
			4 40	85	12	65	43							Died—28 hours	Left bronchopneumonia. Cl. Welchii in legs.
			4 25	125	6	95	55	480	460	− 8	480	480	0	Died—15 hours	Accidental blood loss corrected.
			2 40	130	6 35	35	68							Died—6 hours 45 minutes	
			2 40	100	7 5	55	68							Died—7 hours 35 minutes	
			3 5	110	6	55	54	316	220	−71	293	203	−60	Died—6 hours 40 minutes	
			5 10	105	8	85	62							Died—13 hours	
			5 30	105	7 30	100	31	606	805	+58	430	420	−10	Survived	
			6	80	11	55	48							Died—13 hours	
			3 45	110	6 20	100	34	373	540	+76	221	212	− 9	Survived	
			3	85	9	85	39							Survived	
			6 20	100	8 30	100	37							Survived	
			7 40	130	7 40	130	46							Survived	
			2 20	105	8 40	100	45							Died—24 hours	No water or blood in gut.
			4 20	140	8 15	35	66							Died—8 hours 20 minutes	Empty bladder and intestines Hemorrhage in duodenum.
			7	105	7 50	95	52	555	621	+23	620	620	0	Survived	
			4 30	110	8 30	120	48							Survived	
			10	100	10	100	40	1230	1820	+81	1060	1105	+45	Survived	
			5	110	7 35	100	40	819	730	−20	702	478	−224	Died—11 hours	Empty bladder. Markedly distended gut (2000 ml.+). No blood in gut.
57		2 hours and 4 hours after plasma	9	150	12 20	110	49							Survived	
			7 20	110	13	105								Survived	

* Time from removal of tourniquets.
† Sodium chloride (5 grams per 100 ml.) added to albumin.
‡ Red cell volume determined with radioactive red cells by Gibson and Evans.
× Deviation of the plasma volume found after treatment, from the expected volume calculated on the basis of the intravenously administered fluid—expressed as a percentage of the plasma volume measured in shock.
• 5 per cent sodium chloride solution.
§ First noted time, blood pressure, and hematocrit follow the 5 per cent saline therapy, and the second set of recordings refer to succinic acid therapy (section C).

an occasional dog which died before the 24-hour period succumbed in part to sepsis, since streptococci and *Cl. welchii* were found in the leg muscles of many and in the heart's blood of some of these dogs. Such dogs are listed as dead from shock. At the termination of each experiment, sulfanilamide powder was placed in all wounds before closure.

The therapy given was of 5 types: (*1*) Crystallized bovine serum albumin,[4] given intravenously in 25 per cent solution containing 1.5 per cent NaCl, or in 5 per cent solution containing 0.9 per cent NaCl. (*2*) In certain experiments with 25 per cent bovine albumin, water or

[4] The crystallized bovine serum albumin employed in this work was prepared at the Armour Laboratories, Chicago, Illinois, by the method of Cohn and Hughes, under a contract, recommended by the Committee on Medical Research, of the Office of Scientific Research and Development.

Concentrated human serum albumin was recommended to the armed forces by the Subcommittee on Blood Substitutes of the National Research Council in the interests of convenience for transport and injection. The directions for its use state:

"*Marked dehydration*—As albumin draws fluid into the blood stream at the expense of other body fluids, patients who are severely dehydrated need additional water and salt. This may be given orally, if tolerated, intravenously, or by any other available route."

On each albumin bottle is stamped:

"*Precaution*—In the presence of dehydration, albumin must be given with or followed by additional fluids."

(See Woodruff, L. M., and Gibson, S. T., U. S. Naval Med. Bull., 1942, 40, 791, and Newhouser, L. R., and Lozner, E. L., Ibid., 796.) Insofar as the experimental results herewith reported can be applied to shock in man, the above precaution seems eminently justified.

0.9 per cent NaCl. was given by stomach tube and repeated to the limit of tolerance, *i.e.*, gastric capacity. Where vomiting occurred, the net volume retained is listed as the amount given (Table I). (*3*) Saline solution, 0.9 per cent, intravenously. (*4*) Saline solution, 5 per cent, intravenously, together with water by stomach tube. (*5*) Succinic acid (C.P.) in the doses specified below, dissolved in water by neutralization with NaOH. It was given with or following intravenous bovine albumin or saline solution. A volume of saline solution sufficient to raise the blood pressure above 80 mm. Hg was given when required to ensure as far as possible adequate peripheral vascular distribution of the succinic acid.

RESULTS (*see Table I*)

Group A

As already stated, the almost uniformly fatal shock which most previous investigators report as the result of the application of tourniquets to both legs for a period of 5 hours regardless of the kind of therapy contrasts with our observation that shock is either mild or does not occur providing that no barbiturate or other form of anesthesia except initial doses of morphia are used. In Table I, Group A, 6 dogs, so prepared, are shown to have maintained their blood pressure at generally higher levels and for a longer period following removal of the tourniquets, before any treatment was given, than all other groups listed. Moreover, 5 of the 6 dogs recovered, all 6 having received 0.9 per cent NaCl solution intravenously in varying doses. Only Dog S-72, which died after 22 hours with pulmonary edema, was in deep shock when treatment was started. Two dogs, S-70 and S-71, were apparently not in shock. The other 3 were in mild shock and the response in all but one to saline therapy was quick and adequate in respect to blood pressure and fall of hematocrit. Edema of the legs was considerable in all 6 dogs.

Comment

This group, plus a number of similarly prepared dogs previously observed, convinced us that while 5-hour tourniquet shock *plus barbiturate anesthesia* is a relatively irreversible type of shock, 5-hour tourniquet shock *without anesthesia* is mild and yields readily to saline infusion only.

The successful treatment of tourniquet shock in nembutalized dogs by the use of enormous doses of intravenous saline solution has been reported (9, 10). One view is that a fatal depletion of blood volume results from the increased fluid demands of the damaged tissues (inflammation and increased capillary filtration). If these demands are met by an admittedly nearly fatal volume of saline solution, the depletion of the circulation will stop and recovery from shock will always or nearly always result. Others explain the recovery as due to an increase in pressure of the markedly edematous tissues throughout the body, sufficient to halt the further escape of intravenously injected salt solution (and protein) from the circulation. It is possible however that the recovery may be attributable to the dilution of barbiturate or to the substantial urinary excretion of the barbiturate and the consequent conversion of a relatively irreversible to a readily reversible type of shock.

Group B

The dogs of Group B were prepared like those of Group A except that the tourniquets remained on for 10 hours. They received 0.9 per cent saline solution intravenously in about the same doses as those of Group A and, in addition, succinic acid in an initial dose and by subsequent continuous drip, sufficient to maintain a minimum intravascular concentration of 44 mgm. per cent.[5] The shock was profound in all 6 in less than 3 hours following tourniquet release. All 6 died, 5 in 9 hours or less. In spite of the large volume of saline given, the bladders were empty, showing absence of renal function. Noteworthy is the observation that the tissues were wet in 4, in 4, the lungs were "congested", and in 2, there was hemorrhagic extravasation into the gut.

Group C

The 5 dogs of this group, whose tourniquets were released after 10 hours, received 20 ml. per kgm. of 5 per cent NaCl intravenously to provide an osmotic tension equivalent to that of 20 ml. per kgm. of 25 per cent bovine albumin used in other groups of experiments. Tap water (500

[5] This amount is recommended (11) to provide satisfactory conditions, in a partially or completely anaerobic environment, for maximum utilization of an energy providing substrate which can be burned while glucose cannot be.

to 1200 ml.) by gastric tube was added. The improvement in the shock state was slight and transitory, so that, within 1 to 3 hours, the blood pressures were below 35 mm. Hg. Succinic acid was then given (750 to 850 mgm. per kgm. in 400 to 500 ml. saline) in a large initial dose, followed by continuous drip as in Group B. Improvement was likewise transitory and 4 dogs were dead within 6.2 hours and 1 in 24 hours. Four of the 5 dogs had hemorrhagic bowel, 3 had pulmonary congestion, and 4 had empty bladders.

Comment

We have repeatedly noted the fact that dogs in hemorrhagic or tourniquet shock treated unsuccessfully with intravenous solutions show wet tissues or hemorrhagic intestinal mucosa or both, whereas dogs dying of shock (burn shock excepted) which have received no intravenous fluids do not show wet tissues or hemorrhagic intestinal mucosa. It is our inference that wet tissues are due to a failing capillary circulation which cannot resorb, via the venous end of the capillary, a solution which readily escapes from the arterial end of the capillary and that *lymphatic return of tissue fluids to the circulation* (except perhaps in burns) likewise fails. It is not being argued that wet tissues are due to greater permeability of capillaries to water; rather that the balance between the normal rate of escape and return of water may be upset. The question of disturbed capillary permeability resulting in leakage of plasma was answered in the negative on grounds previously offered (12 to 14), and by the additional evidence herewith provided that, while wet tissues are readily produced by saline infusions, they are less readily produced by infusions of concentrated albumin, whole blood, or plasma. The evidence is that wet tissues are not a phenomenon of shock, but are the result of the fluid therapy of shock and that saline solution is far more prone to produce the effect than whole blood or plasma.

Group D

Seven dogs (tourniquets for 8 hours), when in deep shock, were given 40 to 50 ml. per kgm. of 5 per cent bovine albumin solution in a single transfusion lasting 1½ hours. The dose was equal to two-thirds of the estimated original total plasma volume and may therefore be regarded as adequate, not only to replace loss, but to provide an additional reserve for a continuing loss into the lower extremities. Four received succinic acid, 2 with the albumin and 2 afterwards. Five of the 7 dogs survived. Of these 5, 2 received succinic acid; 3 did not. Both dogs which died also received succinic acid but possibly not in adequate dosage. Their survival was 20 and 28 hours, respectively.

Comment

The evidence is clear that the albumin was therapeutically effective and that the succinic acid probably was not.

Group E

Of 7 dogs which received 5 per cent bovine albumin, as in Group D, but in which supplementary sustaining doses were added as circumstances required, 6 recovered from shock. The seventh was treated only after the blood pressure had fallen precipitously to 30 mm. Hg., *i.e.*, in a preterminal phase. None of these dogs received succinic acid.

Group F

All 5 dogs given 25 per cent bovine albumin in a dose of 10 ml. per kgm. (equivalent to 50 ml. per kgm. of the 5 per cent solution) died. Three of these received succinic acid in saline solution 3 to 4 hours after the albumin had been given and had failed to improve the shock state.

Group G

Two more dogs were treated as in Group F. No succinic acid was given, but saline solution was fed by stomach tube. One died and one recovered.

Group H

All 5 dogs, treated with a dose of 20 ml. per kgm. of 25 per cent albumin and no succinic acid, died.

Group I

Of 6 dogs treated as in Group H but with saline solution by stomach tube, 5 survived.

The sixth was first treated when the blood pressure had dropped precipitously to 35 mm. Hg.

Group J

Of 6 dogs treated as in Group I except that water instead of saline solution was given by mouth, 3 survived. The absorption of water was inadequate in dog B-48. Of the 3 dogs which died, 2 had the lowest blood pressures in the group (50 and 55). These experiments were run on an unusually hot and humid day.

Comment

Whereas the dogs in Group H (25 per cent bovine albumin alone) partly restored the hematocrit toward normal, those in Group I (25 per cent bovine albumin plus peroral saline solution) showed a drop to even lower than normal levels, presumably because saline solution, made available by stomach tube, was effectively absorbed and retained within the circulation. The result was similar to that of Group E (5 per cent bovine albumin), in which the saline was given in almost comparable amounts with the albumin, intravenously.

It appears (*1*) that merely introducing osmotically effective proteins into the blood stream in the absence of a reservoir of fluids upon which to draw for the maintenance of blood volume will not constitute successful therapy; (*2*) that either the tissues lack an adequate fluid reservoir before shock is induced or that this reservoir, however adequate, is pre-empted by the fluid demands of the injured extremities.

The practical effect of these observations, so far as utilization of concentrated plasma and blood substitutes for shock in the field is concerned, is obvious. Supplementary parenteral or peroral fluids must be supplied. Whether the fluid given by mouth should be saline solution or tap water is not settled. In this connection, it should be clear that absorption of fluids from the intestine in shock is deficient (15).

Group K

Two dogs which were given plasma in a dose of 40 ml. per kgm. survived.

DISCUSSION OF RESULTS

It is thus evident that unanesthetized dogs subjected to tourniquet trauma to both extremities for 5 hours will survive if given merely saline solution intravenously (5 survivals in 6 experiments). On the other hand, if the trauma is applied for 8 or 11 hours, all dogs will die whether untreated, treated by intravenous physiological saline solution, or 5 per cent saline solution supplemented with water by stomach tube. The only difference observed in 8 to 11-hour tourniquet shock between the saline treated animals and those not treated was the presence in the former and not in the latter of wet or hemorrhagic tissues (especially the lungs and intestine). It was clear that the saline solution failed to restore renal function, since no urine was excreted during the shock phase in spite of the administration of well over a liter of fluids in each case. The saline solution obviously left the capillaries. Its failure to return in substantial degree to the circulation is evidence of a deficient venous capillary and lymphatic flow. Since hemorrhage in the gut and other tissues was not observed in untreated tourniquet shock, the escape of red cells must also be attributed to intravenous fluid therapy, especially to saline solution. Therefore, when an advanced state of tourniquet shock in the absence of anesthesia exists, the use of intravenous saline solution *alone* must be regarded as having disadvantages without sufficient compensating advantages.

On the other hand, when an osmotically active protein, such as 25 per cent bovine albumin, is given, saline intravenously or orally is in part retained within the circulation. In these circumstances, hemorrhages into the intestine and wet tissues are only rarely seen.

The importance of saline solution as a supplement to protein therapy is shown by the fact that 10 dogs receiving 25 per cent bovine albumin without supplementary saline all died; whereas 5 of 6 dogs receiving supplementary saline solution by stomach tube survived, and 11 of 14 dogs receiving 5 per cent bovine albumin in saline solution also survived.

The characteristically high hematocrit values of tourniquet shock were almost uniformly reduced to normal or less than normal values when therapy was successful. This was as a rule not

the case when therapy failed, whether the therapy was intravenous saline solution alone, 25 per cent albumin solution alone, or 25 per cent albumin and fluid by stomach tube. In the case of intravenous saline solution alone, the insufficiently reduced hematocrit was due to failure of intravascular retention of the infused fluid. In the case of 25 per cent albumin solution alone, it was due to the absence of an available interstitial fluid reservoir, for the 25 per cent albumin solution exerted a satisfactory osmotic effect, as is evident from the extraordinarily dry state of the tissues. When 25 per cent albumin intravenously was supplemented by saline solution via a stomach tube, the saline was effectively absorbed and retained by the circulation, as indicated by the extent of the rise in plasma volume. Thus, in Group H (25 per cent albumin solution alone), dogs B-38 and 41 failed to improve plasma volume above that of the fluid given, whereas in Group J (25 per cent albumin plus saline solution by stomach tube), dogs B-31 and B-33 showed a plasma volume in excess of the expected amount by 25 per cent and 34 per cent, respectively.

A fall in hematocrit to a normal or less than normal level accompanied the increase in plasma volume in 10 instances in which such data were obtained. (The hematocrit change cannot be used to calculate the change in plasma volume.) Eight of these 10 dogs survived.

From the foregoing data, it appears that blood substitutes in the form of plasma, 5 per cent bovine albumin, or 25 per cent bovine albumin plus supplementary fluids by stomach tube, are effective modes of therapy in tourniquet shock. There were 30 dogs which received such therapy. Seventy-three per cent (22 of 30) recovered. In 6 of the 8 which died, therapy was applied at a blood pressure below 60. Only 5 of the 22 which survived received therapy after the blood pressure had dropped below 60. Dogs in tourniquet shock are in an advanced state of collapse when the blood pressure is 70. A mean pressure of 60 may therefore be regarded as close to the permanently irreversible level in tourniquet shock.

The conclusion from the fact that effective replacement of blood volume is curative in tourniquet shock is that this type of shock results from oligemia and is not primarily toxigenic. To what extent toxins liberated from damaged muscle or ischemic tissues play an adjuvant rôle cannot be assessed. That they are of minor consequence is clear (*1*) from the fact that absorption of toxins, if present, is not interfered with, since circulation through the damaged area proceeds satisfactorily, and (*2*) because taping prevents shock by preventing loss of blood volume into the leg, without interfering with absorption. There is, accordingly, no clearly established difference between hemorrhagic and tourniquet shock in respect to the agents responsible for *initiating* the shock state. Necrosis and infection which occur following recovery from tourniquet shock are sequel or adjuvant phenomena bearing no relationship to the rapid collapse immediately pursuant to the release of the tourniquets.

Significant differences between the phenomena of hemorrhagic and tourniquet shock do exist however and these modify the course of events as well as the kind of therapy that is applicable in each. These differences may be tabulated as follows:

	HEMORRHAGIC SHOCK	TOURNIQUET SHOCK
Fluid loss into localized areas	None	Large
Blood deficiency	Whole blood	Plasma—slight volume of red cells
Hematocrit	Normal or low	65 to 85 per cent
Critical level of mean blood pressure	50 to 70 or less and comparatively well tolerated for hours before irreversible collapse occurs	100 or less—levels below 70 poorly tolerated and followed shortly by rapid and irreversible collapse
Relationship of level of blood pressure and its duration to curability	± 20 for hours	Below 40 for minutes
Capillary circulation	Slow flow and capillaries contain few red cells	Slow flow, but capillaries contain many red cells
Sensorium	Dull at blood pressures much below 70	Dull at pressures below 100
Tolerance to blood sampling	Good until very low pressures are reached	Poor at pressures between 80 and 100

The most remarkable physiological differences are the level of blood pressure at which shock may be said to exist (the critical blood pressure), the tolerance to bleeding at low pressures, and the hematocrit level. It is pertinent here to emphasize these differences from the point of view of (*1*) the kind of therapy necessary and (*2*) the time in shock when therapy may still be effective.

Since whole blood will only slightly decrease the hematocrit in tourniquet shock, its use, while perhaps not damaging, would not facilitate as quick a response as plasma or albumin plus saline solution, because the continuing high hematocrit would continue to maintain too high a viscosity for maximum cardiac output.

As already indicated, the blood pressure level is grossly misleading in tourniquet shock. Upon release of the tourniquets, the legs rapidly swell and even though a large loss of plasma has already occurred, the blood pressure tends to remain at some 100 mm. Hg for an hour or two. But the dog is obviously sick. The cardiac output in experiments to be reported (8) was found to be as low as 25 per cent of normal and the venous oxygen 5 volumes per cent within 15 minutes of release of the tourniquets. Once the blood pressure begins to fall, it falls precipitously. To withhold therapy until levels considered reasonably safe in hemorrhagic shock are reached is likely to be disastrous. Therapy at pressures of 100 to 70 however may be expected to be effective.

The relationship between high hematocrit and viscosity is of fundamental significance in the therapy of burn shock, dehydration shock, and other clinical states in which plasma is lost out of proportion to red cells. Data on the extent of the parallelism between viscosity and hematocrit have been gathered (16), as measured by slow and high velocity viscometers and by perfusion of the dog's hind limb. Data by the high velocity viscometer method approximate those of hind limb perfusion. Using the high velocity viscometer method, we have repeated this study on blood from tourniquet shock dogs and correlated the results with the cardiac output. Within the range of clinically encountered hematocrit values, the correlation is one of a progressive fall in cardiac output with a rise in hematocrit (viscosity) providing blood pressure and blood volume are kept constant. The full discussion of this problem will appear in another publication (8). The foregoing may suffice for the immediate purpose of suggesting that the more advanced state of collapse at a given blood pressure and blood volume loss in tourniquet shock as compared to hemorrhagic shock is probably attributable to the high blood viscosity in the former, *i.e.*, that the critical state of the circulation in tourniquet shock is in part due to a qualitative change in the physical properties of the blood itself. Thus the therapy of tourniquet shock must differ from that of hemorrhagic shock in respect to the choice of a substance for blood volume replacement. The problem is one, as in burns, of restoring to normal as rapidly as possible, not only the blood volume, but also the proper proportion of blood constituents.

The effect of sodium succinate on 5-hour tourniquent shock in nembutalized dogs has been studied (17). Significant lowering of mortality was observed. Our data, showing the lack of a positive therapeutic effect of sodium succinate, are not strictly comparable to the above since we employed it in a more severe type of tourniquet shock in the absence of anesthesia. Nevertheless, since we found it possible to cure, by saline solution alone, 5-hour tourniquet shock in which barbiturates were avoided, we regard the therapeutic benefit mentioned above as not related to the shock state *per se*, but to the superimposed depressing effect of the barbiturates. Neutralization of barbiturate action by sodium succinate has been reported (18).

CONCLUSIONS

1. The application of tourniquets to both hind legs of unanesthetized dogs for 5 hours is not always followed by shock. If shock occurs, it is of moderate intensity. Saline solution given intravenously is curative.

2. The application of tourniquets to unanesthetized dogs for 8 to 11 hours will uniformly produce shock which is fatal if untreated. Intravenously administered plasma, 5 per cent bovine albumin in saline solution, or 25 per cent bovine albumin supplemented by peroral fluid are effective therapeutic agents, if the deficiency

in plasma volume is made good while the blood pressure is above 60 mm. Hg. Occasionally, they may be effective at blood pressures between 60 and 40 mm. Hg. Physiologic saline, 25 per cent albumin without peroral fluid, and 5 per cent saline with peroral water are not effective.

3. The critical blood pressure level of tourniquet shock is much higher than that of hemorrhagic shock. This may be related to the deleterious effect on cardiac output of the increased blood viscosity of tourniquet shock. Consequently, the high viscosity requires that plasma or plasma substitutes rather than whole blood be the agent of choice for blood volume replacement therapy.

4. Effective therapy is always accompanied by a substantial reduction in hematocrit and usually by a substantial restoration of the deficiency in plasma volume.

5. The course of events following ineffective though adequate blood volume replacement therapy of tourniquet shock is not materially altered by the administration of sodium succinate. Cure of 5-hour tourniquet shock, attributed by other investigators to succinic acid, is achieved by saline therapy alone in experiments in which anesthesia is omitted. It is therefore apparent that any value sodium succinate may have demonstrated in studies by other investigators may be attributable to its ability to counteract the depressing effects of barbiturates.

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THE EFFECT OF PREGNANCY ON RENAL FUNCTION IN WOMEN WITH PRE-EXISTING ESSENTIAL HYPERTENSION AND WITH CHRONIC DIFFUSE GLOMERULONEPHRITIS [1]

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The effect of pregnancy on the renal function of patients with essential hypertension or chronic glomerulonephritis has been the object of the investigation to be reported here. The study has involved the application of renal clearance methods to a group of patients, during their pregnancies and repeatedly during the 1 to 4 years after their delivery. In 3 patients, the series of records includes also observations made before conception. Such repeated studies were designed firstly to demonstrate the immediate changes in renal function developing during the period of gestation; and secondly, to determine whether, after pregnancy is over, there has occurred any significant acceleration in the course of the primary disease.

These patients with pre-existing renal or vascular disease are to be contrasted with the cases of specific "toxemia" or eclampsia, upon which we have previously published observations. In our first report on this subject, studies based upon inulin and diodrast clearances showed that the glomerular filtration rate, effective renal blood flow, and tubular excretory mass were unaffected in normal pregnancy (1). In a second paper (2), it was shown that in patients with specific toxemia, the glomerular filtration rate was slightly diminished and the effective renal blood flow increased during the latter part of pregnancy. Following delivery, the blood flow became normal in about half of these patients, while in the others it fell to levels indicative of slight renal ischemia. The latter trend occurred in those women in whom hypertension became permanently established after their attack of toxemia of pregnancy. These observations are in general similar to the reported work of other investigators using these methods in cases of toxemia of pregnancy.

Renal function studies of this type have also been made previously in pregnancy complicated by essential hypertension (3, 4). There are, however, no published observations of effective renal blood flow, diodrast Tm, and glomerular filtration rate made before, during, and after pregnancy in the same patient. The studies to be reported are also apparently the first of their kind concerning the effects of pregnancy on chronic diffuse glomerulonephritis.

METHOD

Several aspects of renal function were studied by the clearance principle used in our previous studies. The rate of glomerular filtration was measured at first by the inulin clearance. Later mannitol, whose clearance is identical with that of inulin, was substituted (5). The effective renal blood flow was determined by the diodrast clearance except in the first 2 pregnancies observed in the essential hypertension group. In these, changes in renal blood flow were followed by the phenol red clearance. The latter gives figures which average about two-thirds of the diodrast clearance, but this ratio is not constant and we have not attempted to present actual figures for blood flow computed from the phenol red clearance. Nevertheless, comparison of figures for phenol red clearance in successive observations on the same individual gives a basis for detecting changes in the renal blood flow in that patient. The maximum capacity of the tubules to excrete diodrast was measured by the clearance of diodrast at high levels of plasma concentration. This function, referred to here as diodrast Tm, is regarded as a measure of the mass of functioning tubular tissue (6).

The preparation of the patients for the tests, the collection of the specimens, and the analytical procedures used were those previously described (1, 2). In the cases of this series, as in those already reported, no observation in which the urine flow was below 2 cc. per minute was considered acceptable. Our experience has convinced us that observations made where the urine flow is below this level are of no value in pregnant women, in whom complete emptying of the bladder is especially difficult.

[1] This study was made with the aid of a grant from the Commonwealth Fund.

MATERIAL

Essential hypertension. Observations were made during and after the 7 pregnancies of 6 women suffering from pre-existing hypertension. In 3 of these, who had been patients at the New York University Hypertension and Nephritis Clinic, renal function studies had been made before the advent of pregnancy. After delivery, the patients were followed by further tests giving periods of post-partum observation which varied in duration from 1 to 4 years.

That these were actually cases of uncomplicated essential hypertension and not of primary renal disease is shown by the history of the patients. In none had there been edema, hematuria, or history of acute diffuse glomerulonephritis. In 1 patient whom we have classed in this group, there had been slight proteinuria in addition to the hypertension for 8 years following a pregnancy complicated by eclampsia in 1933. The possibility exists then that this case only should have been classified in the other group. The duration of observed hypertension in these patients before conception is shown in Table I.

Glomerulonephritis. Two women with diffuse glomerulonephritis became pregnant while under observation in the New York University Hypertension and Nephritis Clinic. These patients were known to have exhibited edema, hypertension, proteinuria, and hematuria for several months before their pregnancies began. Urea clearances determined before conception indicated a reduction to about one-half the normal value in one (J. G.) and to two-thirds the normal in the other (P. R.).

Controls. The figures taken as normal for the respective functions studied are made up from the averages obtained from 14 non-pregnant women observed by others (7, 8) and 8 pregnant women observed by us (1). The combination of these groups was considered justifiable since the average figures for the two, the pregnant and non-pregnant women, were practically identical. The following figures, corrected to a surface area of 1.73 square meters, are the averages for the 22 normal women: inulin clearance, 122 ml. per minute; diodrast clearance, 613 ml. per minute; diodrast Tm, 43.7 mgm. iodine per minute; filtration fraction, 19.9 per cent; C_D/Tm_D ratio, 14.0 and C_{IN}/Tm_D, ratio 2.79.

RESULTS

Results are to be considered from two points of view. First, there is the immediate effect of pregnancy on the kidney function of the woman already suffering from chronic renal or vascular disease. Secondly, there is the debated question of whether pregnancy is permanently detrimental to the kidney function of women with one of these diseases. These two aspects must be considered separately in relation to essential hypertension and to chronic glomerulonephritis since current opinion makes a sharp distinction between these two diseases.

The figures for the 48 observations are given in detail in Tables II and III. In these tables, each plasma clearance figure represents the average of 3 urine collection periods; each diodrast Tm figure, the average of 5 urine collection periods.

TABLE I

Clinical data

				Before conception		Pregnancy			Delivery			Postpartum blood pressure range	
	Subject	Age	Parity	Duration of observed hypertension	Blood pressure range	Blood pressure range	Proteinuria	Edema	Date	Week of gestation	Baby	Duration of follow-up	Blood pressure range
1	L. L.	42	4	0		150/94 210/130	0	0	7-24-38	36	Dead	1 mo.	166/105 180/114
2	J. G.	31	1	0		130/90 178/110	0	0	3-11-39	40	Live	1 mo.	148/94 164/90
3	M. J.	37	1	3 years	138/110 170/105	136/98 160/100	0	0	5-19-40	22	Dead	3 years	160/110 240/140
4	M. S.	32	1	0					11- 9-38	40	Live	4½ yrs.	152/102 170/110
		34	2	2 years	144/106 204/118	132/96 164/110	0	0	6- 7-41	40	Live	2 years	130/100 200/120
5	H. F.	28	1	2 years	134/100 192/130	136/100 160/120	0	0	5-12-42	40	Live	1 year	132/100 160/114
6	H. M.	39	4	8 years	180/94 220/130	158/98 220/120	+	+	6- 9-41	40	Live	2 years	160/108 214/130
7	P. R.	34	2	8 mos.	124/92 150/102	120/80 166/114	++++	0	9-13-41	40	Live	2 years	130/94 160/110
8	J. Go.	23	1	2 years	150/110 226/150	160/110 220/150	++++	0	7-29-42	36	Live	1 year	160/120 210/150

L. L. Hypertension noted when the patient was first seen in the second month of her pregnancy.
J. G. Hypertension present when the patient was first seen in the sixth week of her pregnancy.
M. J. Hypertension has persisted since toxemia of pregnancy in 1937.
M. S. Hypertension reported to have been present since 1937.
H. F. Hypertension has persisted since toxemia of pregnancy in 1937.
H. M. Hypertension and proteinuria first noted in 1933, and found at all observations since that time.
P. R. In April 1940, this patient had acute diffuse glomerulonephritis, either an initial attack or an acute exacerbation. Since that time she has had hypertension, proteinuria, and hematuria, and on occasions edema.
J. Go. This patient was first seen in January 1940, in the fourth month of pregnancy, with hypertension, proteinuria, and edema. She was delivered at the seventh month of a premature baby who died. Since that time, she has had hypertension, proteinuria, and hematuria.

Clinical effects of pregnancy on essential hypertension

There are several clinical reports which show that women with essential hypertension are especially liable to develop signs of "superimposed" toxemia if they become pregnant (9 to 11). It was found (12) in 63 per cent of one series of 122 cases that the hypertension was exaggerated at least temporarily during the latter months of pregnancy and in 70 per cent, proteinuria appeared. A report (13) from the New York University Obstetrical Service at Bellevue states that in 59 of 69 pregnancies in women with pre-existing essential hypertension, there developed signs of superimposed toxemia. At the same time, there are certainly cases in which no exaggeration of the hypertension occurs, one report (9) placing the figure for this favorable course at 50 per cent. Indeed, there may actually be a striking temporary decrease in blood pressure during the middle trimester (12, 14). The significance of this is not clear, although it may be ascribed simply to an exaggeration of

TABLE II

Essential hypertension

Subject	Ante-partum or post-partum	Surface area	Date	Duration of pregnancy by history	Time before or after delivery	Blood pressure during test	Proteinuria	Plasma clearances: Inulin	Plasma clearances: Phenol red	Plasma clearances: Diodrast	Effective renal blood flow	Inulin/Phenol red	Filtration fraction: Inulin/Diodrast	Phenol red/Diodrast	Diodrast Tm	Effective renal blood flow/Tm_D A	C_D/Tm_D A	C_{IN}/Tm_D A
		sq. m.		*weeks*	*weeks*			*ml. per 1.73 sq. m. per minute*			*ml. per 1.73 sq. m. per minute*	*per cent*	*per cent*	*per cent*	*mgm. iodine per minute*			
L.L.	A-P	1.76	June 2, 1938	28	8	152/118	0	197	399			49.4						
			June 23, 1938	31	5	170/130	0	198	526			37.7						
			July 14, 1938	34	2	190/114	0	181	661			27.4						
	P-P		August 5, 1938		2	170/100	0	178	461			38.7						
J. G.	A-P	1.73	February 24, 1939	38	2	170/110	0	98	298	460	748	32.9	21.3	68.4	40.1	17.0	10.5	2.23
			March 10, 1939	40	0	158/110	0	103		524	874		19.7		44.9	19.9	11.9	2.34
	P-P		March 21, 1939		2	160/98	0	100	310	378	500	32.3	26.5	82.0	46.6	11.4	8.6	2.28
M. J.		1.53	February 21, 1938*					118	294	485	824	40.2	24.3	60.6	41.1	19.6	11.6	2.81
			March 21, 1938*					132	402	531	848	32.9	24.9	75.7	41.3	20.4	12.7	3.14
	A-P		March 29, 1940	15	7	162/110	0	133	384	610	1046	34.7	21.8	63.0	44.2	24.9	14.5	3.17
			May 15, 1940	22	0	176/114	0	121	339	465	740	35.7	26.0	72.9		17.6	11.1	2.88
	P-P		June 13, 1940		4	180/116	0	92	257	349	498	35.8	26.4	73.7	38.9	11.9	8.3	2.19
			April 4, 1941*			194/120	0	105		461	752		22.8		35.9	17.9	11.0	2.50
			May 7, 1941*			152/98	0	84		570	877		14.7		45.1	20.9	13.6	2.00
			May 24, 1943			208/120	0	89m	312	541	718	28.6	19.8		47.5	17.1	12.9	2.12
M. S.	A-P	1.63	October 18, 1938	37	3	120/80	0	190	535			35.5						
	P-P		November 16, 1938	40	0	204/108	0	138	375			36.8						
			November 21, 1938		1	200/118	0	122	321			38.0						
			December 28, 1938		6	200/128	0	122	205			41.4						
			May 2, 1939			198/118	0	103	257	387	661	40.1	26.6	66.4		12.6	7.3	1.95
	A-P		February 6, 1941	21	19	130/86	0	117	319	624	934	36.7	18.8	51.2		17.7	11.8	2.22
			April 10, 1941	30	10	142/96	0	141	420	706	1025	33.6	19.9	59.5	51.8	19.5	13.4	2.67
	P-P		June 20, 1941		2	126/92	0	104m	363	590	1030	28.7	17.6	61.5	58.4	19.6	11.2	1.97
			January 14, 1942			150/100	0	115m	322	652	1034	35.7	17.7	49.4	54.5	19.6	12.4	2.18
			May 7, 1943			178/108	0	104m	318	448	720	32.7	23.5	71.6	46.0	13.7	8.5	1.97
H. F.		1.44	February 27, 1939			102/134	0	121	319	494	856	37.9	24.5	64.6	42.6	20.1	11.6	2.83
			November 29, 1939			150/106	0	125	329	596	983	38.0	21.0	55.2	36.3	23.0	14.0	2.93
	A-P		January 28, 1942	25	15	130/94	0	131m	420	1006	1444	31.2	12.0	41.7	45.3	33.8	23.5	3.07
			March 26, 1942	33	7	140/100	0	148m	380	672	975	39.0	22.0	56.5	49.2	22.8	15.7	3.47
			May 8, 1942	40	0	196/130	0	103m	304	505	817	33.9	18.2	53.8	44.8	19.1	11.8	2.41
	P-P		May 28, 1942		2	154/100	0	111m	337	598	864	33.0	18.6	56.4	40.1	20.2	14.0	2.60
			June 14, 1943			140/100	0	93m	339	478	787	27.4	19.4	71.0	40.5	18.4	11.2	2.18
H. M.†	A-P	1.82	March 3, 1941	27	13	210/110	+	131		810	1222		16.1		52.1	23.6	15.7	2.53
			March 12, 1941	28	12	180/106	+	136		680	992		20.1		51.2	19.2	13.1	2.63
			March 19, 1941	29	11	190/116	+	122		640	950		19.2			18.4	12.4	2.36
			April 2, 1941	32	8	160/98	+	113		656	965		17.4			18.7	12.7	2.19
			May 12, 1941	37	3	200/120	+	102		584	870		17.4			16.8	11.3	1.97
	P-P		June 16, 1941		1	198/148	+	82		381	584		20.5		55.5	11.3	7.4	1.59
			June 28, 1943			190/110		101m		441	642		22.9		47.9	12.4	8.5	1.95

m Mannitol clearance. Since the mannitol clearance is identical to the inulin clearance, inulin only is used in the headings.

A Average Tm_D figure used.

* Observations made by Goldring, Chasis, Ranges, and Smith.

† H. M. received 475 mgm. of progesterone from March 5 to March 12, 1941.

TABLE III

Chronic diffuse glomerulonephritis

Subject	Ante-partum or post-partum	Surface area	Date	Duration of pregnancy by history	Time before or after delivery	Blood pressure during test	Proteinuria	Plasma clearances: Inulin	Plasma clearances: Phenol red	Plasma clearances: Diodrast	Effective renal blood flow	Inulin/Phenol red	Filtration fraction: Inulin/Diodrast	Phenol red/Diodrast	Diodrast Tm	Effective renal blood flow/Tm_D	C_D/Tm_D	C_M/Tm_D
		sq. m.		*weeks*	*weeks*			*ml. per 1.73 sq. m. per minute*			*ml. per 1.73 sq. m. per minute*	*per cent*	*per cent*	*per cent*	*mgm. iodine per minute*			
P. R.	A-P	1.64	April 17, 1941	15	23	130/86	++++	79	237	392	628	33.4	20.2	60.5	30.7	20.5	12.8	2.57
	P-P		September 23, 1941		2	168/110	++++	67m		424	686		15.8		40.0	17.1	10.6	1.67
			January 22, 1942			140/90	++++			495	800							
			July 12, 1943			130/80	++++	59m	181	319	502	32.6	18.5	56.7	28.0	17.9	11.4	2.10
J. Go.	A-P	1.55	April 9, 1942	25	15	156/114	++++	62m	198	306	504	31.3	20.3	64.7	39.8	12.7	7.7	1.56
			May 15, 1942	29	11	144/100	++++	60m	252	420	708	23.8	14.3	59.9	39.1	18.1	10.7	1.53
			July 2, 1942	36	4	200/135	++++	52m	181	285	473	28.7	18.2	47.0	30.2	15.7	9.4	1.72
	P-P		September 9, 1942		5	200/120	++++	53m	156	260	417	34.0	20.4	59.5	29.8	14.0	8.7	1.78
			July 19, 1943			180/120	++++	48m	144	223	415	33.3	21.5	64.7	26.7	15.5	8.4	1.80

m Mannitol clearance.

the drop in blood pressure which often occurs in normal pregnancy (15 to 17).

The permanent effect of pregnancy on the course of essential hypertension is a separate question, which is greatly confused by difficulties in classification of the vascular diseases of pregnancy. Considerable difference of opinion exists, but the prevailing view appears to be that pregnancy is detrimental to women with essential hypertension. However, it has been found (18) that pregnancy had no ill effects in 52 of 65 patients with pre-existing essential hypertension. Other authors (12) noted that of 89 patients followed for from 6 months to 3 years after delivery, 44 per cent had a blood pressure that was higher than when the patient was first seen, while among 28 patients followed for from 3 to 20 years, 20 had a blood pressure higher than on the first observation. These authors point out further that this incidence of blood pressure increase might be expected during such prolonged periods of observation, irrespective of pregnancy.

In the 7 pregnancies occurring in the 6 patients with essential hypertension in our series, there was no consistent rise in blood pressure, the variations from week to week being no greater than those noted before conception or after delivery, nor were there any other signs of superimposed toxemia. The 7 pregnancies resulted in the birth of healthy normal infants at term with 2 exceptions, each of the latter terminating in the birth of a stillborn premature infant. After labor, the elevation in blood pressure persisted, but there has been no evidence, after periods of post-partum observation of from 1 to 4 years, that the pregnancy has accelerated or otherwise altered the course of the disease.

Renal function in relation to pregnancy with essential hypertension

The clearances of inulin or mannitol were within normal limits in all observations in the group with essential hypertension, whether these were made before, during, or after pregnancy. These patients were evidently at a stage of the disease when glomerular filtration had not yet been affected. Neither the existence of pregnancy nor the after effects of pregnancy produced any alteration of this function.

Diodrast Tm, as studied in 5 of these patients, was also found to be normal throughout and thus likewise unaffected by the primary disease or by pregnancy or parturition.

The effective renal blood flow, as measured by the clearance of diodrast in 5 pregnancies and indicated by the phenol red clearance in the other 2, was found to be subject to what appear to be significant variations. Details of these tests may be seen in Table II. The changes in the C_D/Tm_D ratio, giving the plasma flow per unit of excretory tubular tissue, can be seen more readily in Figure 1.

Before conception, the figure for the renal blood flow tended to be low in the 3 patients upon

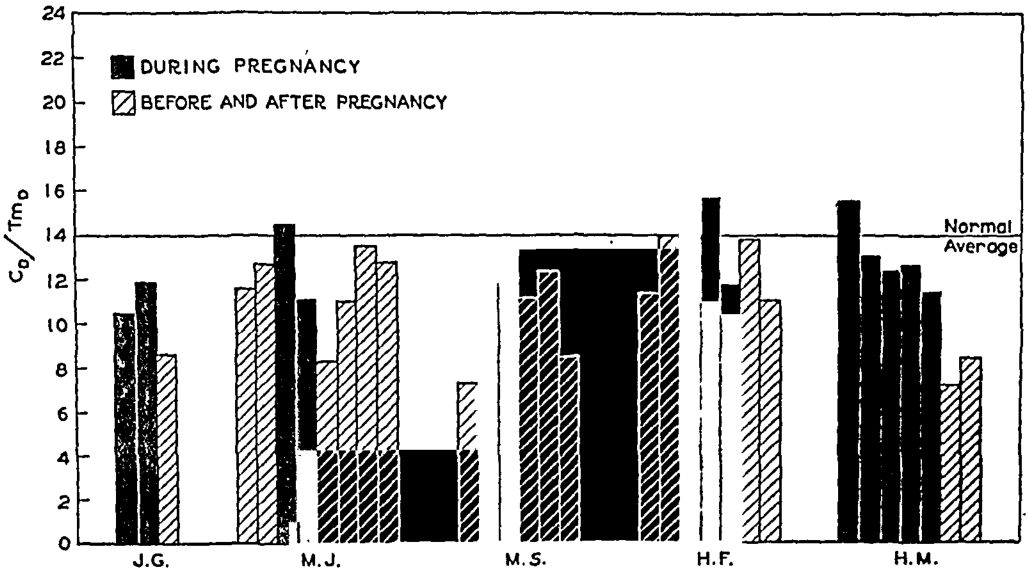

FIG. 1. CHANGES IN RENAL PLASMA FLOW PER UNIT TM BEFORE, DURING, AND AFTER PREGNANCY IN WOMEN WITH ESSENTIAL HYPERTENSION

whom observations were made at this time. Figures indicating a reduced renal blood flow are of course characteristic of patients with essential hypertension (8). In each of these, the diodrast clearance rose during their subsequent pregnancy.

After all of the 5 pregnancies studied by diodrast clearances, the blood flow was found also to be somewhat below normal. In each of these cases, there had been a higher average diodrast clearance and higher figures for the C_D/Tm_D ratio (Figure 1) during their pregnancies than after their delivery. In 2 additional pregnancies studied only by the phenol red clearance, there was also found to be a higher figure for this function during pregnancy than after delivery. It may be significant that in 3 of the 7 pregnancies studied, the renal blood flow had fallen somewhat in the last observation made before delivery so that had these studies been limited to the last 3 weeks of pregnancy, the now apparent increase in blood flow during pregnancy might have been overlooked.

The filtration fraction (C_{IN}/C_D) and the inulin/phenol red clearance ratio are affected by these changes in renal blood flow. In general, these ratios were found high before conception and after delivery but approached the normal figure during pregnancy. On the basis of the significance placed upon the filtration fraction by some authors (20), the decrease in the filtration fraction which develops when hypertensive women become pregnant may be attributed to a diminished tone of the efferent arteriole from the glomerulus.

In the 3 patients in whom diodrast clearances were done both before and after pregnancy, there was no evidence of any diminution in renal blood flow as a result of the pregnancy. In 4 cases in which a series of clearance tests were made during a follow-up period, ranging from 1 to 4 years after delivery, there was no evidence of any progressive deterioration of renal function after pregnancy.

Clinical effects of pregnancy on chronic diffuse glomerulonephritis

The effect of pregnancy on chronic diffuse glomerulonephritis is considered by most obstetricians to be worse than on essential hypertension. One author (14) writes that it is unusual for a patient with chronic diffuse gomerulonephritis to give birth to a live baby at term, or even for the pregnancy to continue to term. Others (18) believe that about half of their 17 patients with this disease became worse as a result of their pregnancies. A somewhat more optimistic report has been given (12) on the basis of 15 cases. Among these, the blood pressure increased during pregnancy in 8 and the proteinuria increased in 7. From 6 months to 5 years after delivery, 4 patients were worse, 2 being in a serious condition. The usual course of the disease, uncomplicated by pregnancy,

might, however, give results not dissimilar from these.

The 2 patients with chronic diffuse glomerulonephritis in our group completed their pregnancies and were delivered at term of normal children without increase of either their hypertension or proteinuria. Twenty-eight months have elapsed since the delivery of 1 of these patients and 16 since that of the other. Both continue to show evidence of active glomerulonephritis with hypertension, proteinuria, and hematuria, but in neither is there evidence of significant progression of the renal lesion as the result of the pregnancies.

Renal function in relation to pregnancy and chronic glomerulonephritis

The 2 patients with chronic glomerulonephritis were known to have had diminished urea clearances before conception. At the time they were first examined by the more complete clearance methods, they were already pregnant. In each, there were then noted abnormally low values for the glomerular filtration, renal blood flow, and tubular excretory mass. That the glomerular filtration rate and renal blood flow were impaired to a greater extent than the tubular excretory mass can be seen from the low values of C_{In}/Tm_D and of C_D/Tm_D.

On the basis of only 2 cases, no consistent difference can be pointed out between antepartum and post-partum values, so that in this disease pregnancy appears to have had no effect on renal blood flow. This, in distinction to the alterations noted in cases of essential hypertension, might be due to the more permanent character of the changes causing disturbances in function in the kidney with chronic nephritis.

When comparing the values obtained in the observations made during pregnancy with those made 1 and 2 years, respectively, after the delivery of the 2 women, one finds a suggestion of slight diminution in all functions measured. These changes are not striking, perhaps not significant, but are consistent with the slowly progressive course which is usually characteristic of the disease. There is no evidence, however, of an abrupt change in the course resulting from pregnancy.

DISCUSSION

Two points are of special interest. First, it is necessary to consider the significance of the apparent increase in renal blood flow which developes when the woman with essential hypertension becomes pregnant. Secondly, consideration must be given the clinical implications of the renal function tests made before, during, and at intervals after a pregnancy complicated by pre-existing renal or vascular disease.

The increase in the renal blood flow during pregnancy, as indicated by the diodrast and phenol red clearance, appears to be real, although slight in degree. In each of the 3 patients upon whom diodrast clearance observations were made before conception, the figures for blood flow were found to have risen during pregnancy. Similarly, in all 7 patients, it could be shown that the blood flow fell after delivery.

This behavior of the renal blood flow in cases of essential hypertension is parallelled by observations made on patients with specific toxemia (2). In the latter condition, the renal blood flow is normal or slightly elevated during pregnancy, but in those patients who develop a persistent hypertension, evidences of relative renal ischemia appear immediately after parturition.

These results may have some bearing on the effects of pregnancy upon blood pressure. In experimental animals, previously rendered hypertensive by constriction of the aorta or renal artery, there has been observed a decrease in the level of hypertension with the advent of pregnancy (21 to 25). Furthermore, if the operation for the reduction of the renal blood flow is carried out after pregnancy has commenced, the rise in blood pressure is frequently delayed till after delivery (25).

No evidence can be found in a review of the records of our patients of even a transitory decline in blood pressure. Other observers (12) have noted, however, that in a considerable proportion of patients with essential hypertension there occurs a rather "striking" decrease in the blood pressure during the middle trimester of pregnancy. The opposite effect, namely a temporary rise in blood pressure for several weeks after the conclusion of a normal pregnancy, has recently been described with reports of a considerable number of such cases (27, 28).

Speculation on the cause of the improved renal blood flow noted in pregnant hypertensive patients leads at once to a consideration of the placenta. The sudden contraction of the total vascular bed through the elimination of the large circulation of the maternal portions of the placenta might conceivably affect blood pressure for a short time but it is difficult to see how renal circulation could be affected by this means. Evidence that any hormones of placental origin have a direct effect on renal blood vessels has not been produced, but it is known that the estrogens and perhaps other sex steroid hormones have some effects on the peripheral circulation (29). It is at least possible that the blood flow of the kidney in hypertensive pregnant patients is maintained by some such substance from the placenta whose loss at delivery is at once followed by relative renal ischemia.

The failure to find signs of any deterioration in renal function after pregnancy in women with essential hypertension or chronic glomerulonephritis is a point of practical importance in the handling of these patients. These observations run somewhat contrary to existing beliefs.

Satisfactory statistics showing the effect of pregnancy on pre-existing essential hypertension and chronic glomerulonephritis are meagre and confusing due to lack of agreement on the criteria for classifying cases. It is agreed that an attack of specific toxemia of pregnancy may have chronic vascular disease as a sequela and that in cases of essential hypertension, a "superimposed" toxemia is liable to occur. It may be inferred that an attack of toxemia in a patient already suffering from essential hypertension or chronic glomerulonephritis will be detrimental. However, it is also evident that patients with these diseases may go through their pregnancies without added complications and our studies indicate that pregnancy without superimposed toxemia has no permanent effects on the renal function of women suffering from chronic vascular or renal diseases.

CONCLUSIONS

1. Renal function studies made upon patients with essential hypertension indicate that pregnancy is associated with a slight temporary increase in renal blood flow. Glomerular filtration rate and the tubular excretory mass (Diodrast Tm) are unaffected by pregnancy in these women.

2. Comparison of results obtained during pregnancy and for an observation period of 1 to 4 years after delivery indicate that pregnancy itself, when uncomplicated by specific toxemia, does not cause any deterioration of renal function in women with essential hypertension or chronic glomerulonephritis.

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CHANGES IN RENAL FUNCTION ACCOMPANYING THE HYPERTROPHY OF THE REMAINING KIDNEY AFTER UNILATERAL NEPHRECTOMY [1]

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The functional changes which accompany the hypertrophy of the kidney remaining after unilateral nephrectomy have been followed by the measurement of the glomerular filtration rate, effective renal blood flow, and tubular excretory mass. Such observations may serve to demonstrate the mechanism by which hypertrophy is accomplished.

That hypertrophy of the remaining kidney after unilateral nephrectomy occurs, and that this hypertrophy is not accomplished by the formation of new nephrons, have been demonstrated repeatedly (1 to 6). It has been suggested that this organ growth is a work hypertrophy, a response to functional demands. This view is supported by the fact that the degree of growth is greater on high than on low protein diets, and by the fact that the larger the proportion of total renal substance resected, the greater is the relative hypertrophy (6 to 12).

Changes in renal function, comparable to the increase in kidney weight, have not previously been demonstrated. However, the great increase in diodrast Tm in the dog following the administration of testosterone propionate (13), a substance known to increase kidney weight in rats and mice (14 to 17), certainly suggests that renal hypertrophy is accompanied by an increase in diodrast Tm. The increase in diodrast Tm in the dog following the administration of testosterone propionate may be as much as 100 per cent (13). A very much smaller increase in diodrast Tm in man has been shown when testosterone propionate was administered soon after unilateral nephrectomy, and no increase when given to men with both kidneys present (18).

[1] This study was made with the aid of a grant from the Commonwealth Fund.

The present communication reports the observations on renal function, made after unilateral nephrectomy performed during the pregnancies of 2 women. Studies were made within a few weeks of the operation and were repeated at intervals until 14 and 40 months had elapsed, respectively. Renal function was measured by the clearance method. The clearance of inulin or mannitol has been shown to be a measure of the rate of glomerular filtration; the diodrast clearance, of the amount of blood supplying excretory tissue. The diodrast Tm is an expression of the maximal capacity of the tubules to excrete diodrast and it has therefore been considered a measure of total functioning tubular tissue (19, 20). The methods of performing the tests were described in a previous report (21). The normal control group with which these subjects with a single kidney are compared consists of 14 non-pregnant women (22, 23), and 8 pregnant women (21). The average figures for the control group are: inulin clearance 122 ml. per minute, diodrast clearance 613 ml. per minute; diodrast Tm 43.7 mgm. iodine per minute, filtration fraction 19.9 per cent, C_D/Tm_D ratio 14.0, and C_{IN}/Tm_D ratio 2.79.

CASE HISTORIES

The histories of the 2 patients are to be contrasted in one respect in particular. In the first patient, the removed kidney was apparently a functioning organ at the time of the nephrectomy, while in the second, the kidney had already undergone almost complete destruction as a result of a malignant tumor.

1. E. M. was a 29-year-old colored female whose left kidney was removed at the sixth month of her pregnancy. This patient had suffered for 3 years from painless hematuria and repeated cystoscopic examinations had shown bleeding from the left ureteral orifice. The only renal function test before operation was the timed appearance of

methylene blue from the 2 ureters, which was found to be normal.

Recovery from the operation was uneventful and pregnancy proceeded normally until the ninth month. At that time, she rather suddenly developed specific toxemia with hypertension, proteinuria, convulsions, and coma and was delivered of a dead fetus at another hospital. Following delivery, the blood pressure reached normal on the eighth post-partum day, but 1 month after delivery was again high. The blood pressure, next taken 2 months after delivery, was normal and has remained so at each of the frequent observations in the last 3 years.

The pathological findings in the kidney removed at operation have been reported in detail (24). Multiple hemorrhagic erosions of the pelvis and ureter were present to explain the persistent bleeding. In addition, there were massive medial hypertrophy and hyperplasia of the arterioles, a finding most prominent on the afferent side of the glomerulus. The architecture of the kidney was well preserved, the glomeruli and tubules themselves appearing normal.

2. M. C. was a 40-year-old white female whose right kidney was removed in the fourth month of her sixth pregnancy. Recovery from the operation was uncomplicated. The patient was delivered at term of a normal infant.

Pathologic examination of the removed kidney disclosed practically no normal renal parenchyma, all having been replaced by adenocarcinoma. The patient has recently been examined and there has been to date no evidence of any recurrence.

RESULTS

The functional compensations which develop after nephrectomy may be followed by noting the changes in the clearance measurements which are given in detail in Table I. The essential points to be noted are as follows:

1. *Effect of removal of a functioning kidney (Case E. M.)*

Sixteen days after nephrectomy. At the time of these measurements, the patient was beginning the seventh month of her pregnancy and had clinically recovered from her operation. The diodrast Tm was one-half the average normal value, the clearances of inulin and diodrast about three-fourths the normal value. The ratios of the glomerular filtration rate and of effective plasma flow to the tubular excretory mass (C_{IN}/Tm_D and C_D/Tm_D) were therefore relatively high. The figure of 19.3 for the C_D/Tm_D ratio is above the normal mean by more than 3 times the standard deviation of this figure in the control group and demonstrates the presence of hyperemia in the remaining kidney.

Four months after nephrectomy. At this time, the patient was 1 month post-partum after a pregnancy complicated by specific toxemia and her blood pressure was still elevated. All post-partum patients with residual hypertension after toxemia in our experience (25) have shown a tendency to renal ischemia.

In the present case, the diodrast Tm was found but little higher than the level noted immediately

TABLE I

Renal clearances following the removal of a functioning and a non-functioning kidney

Subject	Date	Time before or after delivery	Time after nephrectomy	Blood pressure during test	Diodrast Tm	Plasma clearances: Inulin	Plasma clearances: Phenol red	Plasma clearances: Diodrast	Effective renal blood flow	Inulin/Phenol red	Filtration fraction Inulin/Diodrast	Phenol red/Diodrast	Effective renal blood flow/Tm_D	C_D/Tm_D	C_{IN}/Tm_D
					mgm. iodine per minute	*ml. per 1.73 sq. m. per minute*			*ml. per 1.73 sq. m. per minute*	*per cent*	*per cent*	*per cent*			
E. M.	March 11, 1940	A 3 mos.	16 days	120/70	23.2	92.	246	446	718	37.4	20.6	55.2	31.0	19.2	3.97
	June 26, 1940	P 1 mo.	4 mos.	162/100	26.0	73.	202	278	465	36.2	26.3	72.6	17.9	10.7	2.81
	October 2, 1940		7 mos.	118/78	25.8	98.	259	423	745	37.8	23.2	61.2	28.9	16.4	3.80
	February 27, 1941		12 mos.	120/70	33.3	79.	272	564	1040	29.0	14.0	48.2	31.2	16.9	2.37
	October 28, 1941		20 mos.	110/70	42.6	73.[m]	260	565	1017	28.1	12.9	46.0	23.9	13.3	1.71
	September 23, 1942		31 mos.	134/84	34.2	81.[m]	263	600	952	30.8	13.5	43.8	27.8	17.5	2.37
	July 26, 1943		40 mos.	120/80	33.6	79.[m]	219	440	697	36.0	18.0	49.8	20.7	13.1	2.35
M. C.	November 17, 1941	A 2 mos.	2 mos.	140/86	34.4	70.0[m]	261	344	525	26.8	20.3	75.9	15.3	10.0	2.03
	January 7, 1942	A 3 wks.	4 mos.	134/90	31.2	69.0[m]	216	371	533	32.0	18.6	58.2	17.1	11.9	2.21
	February 2, 1942	P 6 days	5 mos.	136/88	39.3	74.0[m]	211	451	678	35.1	16.4	46.8	17.3	11.5	1.88
	May 21, 1942		8 mos.	120/80	36.9	62.0[m]	221	483	737	28.1	12.8	45.8	20.0	13.1	1.68
	November 18, 1942		14 mos.	110/70	34.5	65.0[m]	187	340	536	34.8	19.1	55.0	15.5	9.86	1.88

m Mannitol clearance. Since the mannitol clearance is identical to the inulin clearance, inulin only is used in the headings.

after nephrectomy. The diodrast clearance was diminished and the filtration fraction correspondingly increased to the levels believed to be characteristic of efferent arteriolar hypertonus (26). The functional changes associated with the post-toxemic hypertension appear at the time of this test to overshadow those due to the compensatory alterations in the kidney remaining after nephrectomy.

Seven months after nephrectomy. At this time, the patient had ceased to show any sequellae from the eclampsia and her blood pressure was normal. The results at this time were almost identical with those found in the first test. The diodrast clearance had risen to the level seen before the advent of toxemia and renal hyperemia was again evident.

Twelve months after nephrectomy. At the end of 1 year, the diodrast Tm had increased from the original figure of 53 per cent to 76 per cent of normal. The diodrast clearance had continued to increase, so that renal hyperemia was evidently still present. The inulin clearance on the other hand had decreased slightly and the filtration fraction had fallen to the lower limits of normal.

Twenty months after nephrectomy. The diodrast Tm for the remaining kidney had now increased still further and was found only 3 per cent below the average normal figure for 2 kidneys. This figure is within the normal range and the compensatory hypertrophy may thus be thought of as complete. The diodrast clearance had previously reached correspondingly high levels and the blood flow to the excretory tissue was therefore now normal. With the exception of the low rate of filtration at the glomerulus, the 1 kidney was now doing the amount of work normally done by 2 kidneys.

Thirty-one months after nephrectomy. In this observation, a fall in diodrast Tm was noted, with no change in glomerular filtration rate or effective renal blood flow.

Forty months after nephrectomy. The diodrast Tm showed no further change. The effective renal blood flow was lower than that shown by any observation from the twelfth through the thirty-first month after operation, indicating that hyperemia was no longer present. However, the blood flow per unit of excretory tissue and the filtration fraction were normal.

2. *Effect of removal of a non-functioning kidney (Case M. C.)*

At the time of the first test, made 2 months after nephrectomy, the patient was in the eighth month of a normal pregnancy. Observations made at this time showed the diodrast Tm to be about three-quarters of the normal average while the inulin and diodrast clearances were little more than half the normal.

In subsequent measurements, at 4, 5, 8, and 14 months after nephrectomy, the diodrast Tm showed no progressive change. The diodrast clearance exhibited a slight but consistent increase through the eighth month. The inulin clearance, however, remained low, but without significant differences, in the 5 tests. The ratio of glomerular filtration rate to tubular excretory mass (C_{IN}/Tm_D) was low in all of the observations, and that of the effective plasma flow to the tubular excretory mass (C_D/Tm_D) low except in the one carried out in the eighth month after operation. At no time then was there evidence of the renal hyperemia noted in the first case.

DISCUSSION

There is little reason to suppose that pregnancy had any special bearing on the changes observed following nephrectomy in either case. Normal pregnancy has no effect on the renal function measurements that were used here (21), and at the time of the earliest observations on each case, both women, though pregnant, were normal. In the first patient, a transitory episode of hypertension following eclampsia was accompanied by a temporary reduction in the renal blood flow. In all other tests subsequent to the pregnancies of both women, there were no signs or symptoms of vascular or renal disease.

The results obtained in the study of the 2 patients offer a marked contrast which is to be explained on the basis of the functional capacity of the kidney removed.

After the loss of an active kidney, the functional changes which occur are probably as follows: The tubular excretory mass must at once be cut in half as a result of the anatomical re-

moval of half of the renal tissue of the body. The glomerular filtration rate and effective renal blood flow are, momentarily at least, reduced to a similar extent. Very soon, however, as a result of functional changes, the glomerular filtration rate and the effective renal blood flow increase considerably.

The early increase in glomerular filtration rate is apparently the maximal expansion of this function under these conditions but the effective blood flow continues to increase for several months. This may be accomplished, in part at least, by efferent arteriolar dilatation which would result in a lowered intraglomerular pressure and thus account for a decreased filtration rate.

The tubular excretory mass does not increase at first as rapidly as does the effective renal blood flow and during this early period, there is a relative hyperemia of the renal excretory tissue. This hyperemia persists until the time when the compensatory hypertrophy of the tubular excretory tissue has been accomplished. In the first case studied, the completion of this process apparently required nearly 2 years.

The observable effects of the loss of a nonfunctioning kidney are quite different, since the early changes must necessarily have taken place some time before nephrectomy. The kidney removed at operation in the second of our patients contained, as noted, no normal renal tissue. The figure for the tubular excretory mass 2 months after operation was already three-fourths of the average for 2 normal kidneys, a value which was not reached by the first patient until a year after her operation. Renal hyperemia was furthermore not evident in any of the observations made on the second case, despite the slight rise in effective blood flow notable through the eighth month.

CONCLUSIONS

1. The functional changes accompanying the renal hypertrophy which follows unilateral nephrectomy have been studied in 2 patients.

2. Following the removal of a previously functioning kidney, there was evidence of a rapid rise in glomerular filtration rate and effective renal blood flow and a much delayed increase in the mass of functioning tubular tissue (diodrast Tm). The increase in diodrast Tm apparently parallels the morphologic hypertrophy of the kidney.

3. Following the removal of a kidney previously destroyed by disease, these changes were not noted. The high figure for the diodrast Tm demonstrated, however, that a similar hypertrophy must have occurred previous to operation in this case.

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ABSORPTION OF FERROUS AND FERRIC RADIOACTIVE IRON BY HUMAN SUBJECTS AND BY DOGS[1]

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Much evidence has been accumulated to indicate that human subjects absorb ferrous more readily than ferric iron. There is agreement among physicians and clinical investigators that patients with hypochromic anemia respond with maximal hemoglobin production to amounts of soluble ferrous salts containing only one-fourth to one-sixth the quantity of iron which must be given in the form of a soluble ferric salt to produce comparable therapeutic effects (1 to 10). Furthermore, studies of iron absorption have regularly shown that the bivalent form of the metal is more completely assimilated (11 to 13). In studies made on experimental animals, however, results have been less consistent. Many investigators have found that laboratory animals absorb both valence forms of iron equally well (14 to 18), several claim superiority for ferric iron (19, 20), while others have either observed greater assimilation of the ferrous form or have claimed that only ferrous iron was absorbed (21 to 25). This lack of agreement in the animal studies may be traced in part, at least, to the different methods used for measuring absorption and to the inadequacy of several of the technics. One is impressed by the fact that the workers in the two laboratories in this country which have studied iron metabolism in animals most intensively are convinced that there is no difference in the degrees to which ferrous and ferric salts are absorbed (14 to 16).

Consequently, a disagreement has resulted between the clinical and some of the animal investigators as to the form in which iron is taken up by the body from the intestinal tract. There are two possible explanations for this difference of opinion: either one group of workers is in error, or else both are correct and animals absorb ferric iron more efficiently than do human subjects. With the development by Hahn and his associates of an accurate method of measuring iron absorption by using the radioactive isotope of iron, it is now possible to determine experimentally which of the two explanations is correct. This communication describes such studies made on (*1*) normal and iron-deficient dogs, and (*2*) normal and iron-deficient human subjects. Dogs were selected because in these animals Whipple, Hahn, and their associates have "never observed any significant difference in iron absorption whether the metal was given as a ferric or ferrous salt" (26). The data reported below demonstrate that human subjects almost always show significantly greater assimilation of radioactive ferrous salts than of ferric, whereas dogs frequently absorb both valence forms equally well.

I. METHODS

The validity of the technic for quantitating the amount of iron absorbed from a given dose of radioactive iron has been established by demonstrations that (*1*) the absorbed iron is rapidly synthesized into hemoglobin and appears in the peripheral blood (26 to 29) where its radioactivity can be measured; and (*2*) excretion of the absorbed iron during the period of study is minimal (30, 31). We have been able to confirm the first of these demonstrations. Our curves for the appearance of radioactive iron in the red cells are comparable to those of other investigators. The use of the method also requires acceptance of the postulate, true so far as is known, that the physiological properties of a radioactive isotope are identical with those of the stable form of the element (32). The assumption that the amount of the isotope which appears in the peripheral blood represents the total amount absorbed does not introduce a large error: that portion which remains in the tissues is not easily determined, but it appears to be small (27).

The human subjects used in this study were: (*1*) healthy male medical students or laboratory workers who had suffered no known blood loss and who had not served as donors for transfusions; and (*2*) patients in the Barnes Hospital who volunteered as subjects. The animals were mongrel dogs, kept in individual metabolism cages and fed either the salmon bread diet of Whipple (33) or Purina

[1] These studies were made with the help of grants received from Mr. Edward Mallinkrodt, Jr., and the John and Mary R. Markle Foundation.

dog chow. Four dogs were depleted of their iron reserves by systematic bleeding, 3 times a week for several months. During the period of depletion, they were fed either the salmon bread diet or milk and bread supplemented with brewer's yeast or a vitamin B-complex concentrate.

The radioactive iron (Fe^{59}) used in the experiments was prepared in the Washington University Cyclotron by deuteron bombardment of iron.[2] By using a probe prepared by electroplating a thin layer of iron on copper, radioactive iron of very high specific activity was formed. Because considerable amounts of radioactive copper, cobalt, and manganese are formed during the bombardment, precautions were taken to purify the iron and free it of these other radioactive substances. The iron was dissolved by dipping the copper probe in concentrated HCl; the solution was then diluted so that the acid concentration was about 1 N, the copper was precipitated with H_2S, and the precipitate was removed by filtration. The filtrate was next heated and, if no further cloudiness appeared, boiled to expel the H_2S. The iron in the filtrate was re-oxidized to the ferric state by the addition of 30 per cent H_2O_2 to the hot solution. It was then precipitated with ammonium hydroxide. The $Fe(OH)_3$ was thrown down by centrifugation and dissolved in 6.4 N HCl. Small amounts (20 mgm.) of inert $CoCl_2$ and $MnCl_2$ were added as carriers. Subsequent purification was accomplished by either of the following 2 methods, the second of which has been used in the more recent experiments. Both methods were apparently equally satisfactory.

1. The iron was extracted from the 6.4 N HCl solution by continuous extraction with ethyl ether. The ether extract was thoroughly washed with 6.4 N HCl to remove traces of cobalt and manganese which had been carried over into the ether layer. The ether extract was then evaporated to dryness and the $FeCl_3$ taken up in HCl.

2. The iron was purified by repeated precipitation with pyridine (34). Before each precipitation, 20 mgm. each of $CoCl_2$ and $MnCl_2$ were added as carriers. Precipitations were made until the supernatant solution showed no activity when measured with a Geiger-Müller counter. The iron was then taken up in HCl. The freedom of the purified radioactive iron solution from radioactive elements other than the 47-day iron was confirmed by the shape of the decay curve.

The dose of iron given varied from 1 to 4 mgm. per kgm. of body weight and was administered after a 12-hour fast. Dogs were fed the material by stomach tube. Precautions were taken to have all of the iron of a given dose in the state of valence desired. The solution of radioactive ferric chloride was heated and oxidized with 2 drops of concentrated nitric acid, then cooled, and partially neutralized with sodium hydroxide to a pH of about 3. The solution of ferrous salt was prepared by reducing the ferric salt with ascorbic acid, immediately before it was given.

Samples of dog blood were obtained by puncture of one of the femoral arteries; specimens from the human subjects were obtained by venepuncture in the usual manner. The red cells were thrown down by centrifugation and were digested in a Kjeldahl flask with concentrated H_2SO_4 and 30 per cent hydrogen peroxide. The iron was precipitated from the digest as ferric hydroxide, in the manner described by Hahn (27). After several reprecipitations, the iron hydroxide was dissolved in 0.2 cc. of 6 N HCl and was electroplated from a bath of 3 parts saturated ammonium oxalate solution and 1 part saturated oxalic acid solution, at a current of 1 ampere. The anode was a strip of platinum; the cathode was a copper disc to which had been soldered a brass stem. In order to have all the iron electroplated on the face of the copper disc, a disc of bakelite (with a center hole to slip over the brass rod) was cemented to the back with Glyptal.[3] The brass rod was also painted with Glyptal to a distance of 1 cm. from its junction with the bakelite disc. During the electroplating, the cathode was rotated just below the surface of the solution by an electric stirrer. In order to get a firm deposit of electrolytic iron, 2 to 5 mgm. of inert iron as ferric chloride were added to the iron from each blood sample. Electroplating was continued until the bath became iron-free, as determined by testing with ammonium sulfide solution. This generally required 2 hours. The method is similar to the one described by Ross and Chapin (35).

Radioactivity measurements were made with a Geiger-Müller counter having a scale of 8. A dip type of Bale tube was used at first with the iron dissolved in exactly 2 cc. of concentrated HCl (36), but soon after the observations were begun, change was made to a more sensitive bell-shaped counter tube with a mica window.[4] The iron, isolated from the red cells and electroplated on a copper disc of the same diameter as the window, was placed in a fixed position immediately below the window of the counter tube. By using this technic to replace a glass-walled dipping type of counter, a 30-fold increase in sensitivity was gained.

In addition to the radioactivity measurements, red blood cell counts were made with equipment standardized by the U. S. Bureau of Standards, hemoglobin determinations were made with the Evelyn photometer, hematocrit values were obtained in Wintrobe tubes centrifuged for 20 minutes at 3,000 r.p.m.

When the percentage of the dose absorbed was calculated, the blood volume was arbitrarily assumed to be 80 cc. per kgm. This assumption was probably no more inaccurate, as far as the red cell volume is concerned, than would have been an estimation of the blood volume by the standard dye procedures (37).

It will be observed that the figures given for the percentage of absorption refer to a single dose of iron, either in the

[2] The first two samples of iron used were supplied through the courtesy of Dr. Ernest Lawrence and Dr. M. Kamen of the University of California.

[3] Made by the General Electric Company.

[4] A helium-filled counter has been developed by Dr. Arthur Kip and Dr. Robley Evans of the Massachusetts Institute of Technology. We are indebted to them for supplying us with these tubes. Dr. Alex Langsdorf, Jr., of Washington University, has also furnished us with a bell-type counter filled with argon and alcohol which has a high sensitivity.

TABLE I

Absorption of ferric and ferrous iron by normal dogs

Dog	Date	Wt.	R.B.C.	Hgb.	Cell vol.	Dose of Fe	Valence of iron	Radioactivity	Labeled iron in blood stream*	Ratio of amount of Fe^{++} to Fe^{+++} absorbed
		kgm.	*millions*	*grams*	*per cent*	*mgm. per kgm.*		*counts per min. of total dose*	*per cent*	
32	6/ 8/43	33	6.15	15.3	46	1	Fe^{+++}	10,800,000	1.9	
	6/23/43		5.72	13.8	43	1	Fe^{++}	8,775,000	5.9	3.1
	8/13/43		6.41	16.8	49	1	Fe^{+++}	7,212,000	6.0	
	8/25/43	32.2	6.56	17.0	48	1	Fe^{++}	6,300,000	5.0	0.8
33	8/27/43		5.98	16.5	47	1	Fe^{++}	4,160,000	0.8	
	9/10/43	16.4	5.76	14.0	40	1	Fe^{+++}	3,290,000	0.3	2.7
34	8/27/43		6.51	15.8	48	1	Fe^{+++}	4,180,000	1.3	
	9/10/43	11.8	6.60	15.8	45	1	Fe^{++}	3,280,000	7.8	6.0
	9/28/43		6.21	15.8	46	1	Fe^{+++}	1,378,000	7.5	
	10/ 8/43		6.20	16.1	45	1	Fe^{++}	1,226,400	8.8	1.2
38	10/22/43		6.35	14.3	44	1	Fe^{+++}	976,000	1.1	
	11/ 5/43	12.7	5.64	13.8	43	1	Fe^{++}	848,000	6.3	5.7
	1/ 7/44		7.18	15.8	48	1	Fe^{++}	1,992,000	7.6	
	1/25/44		7.30	16.5	49	1	Fe^{+++}	1,512,000	3.7	2.1
41	12/31/43		5.40	16.0	47	1	Fe^{+++}	2,945,000	2.7	
	1/25/44	12.3	5.26	14.5	43	1	Fe^{++}	2,016,000	5.0	1.8
44	12/31/43		5.73	15.8	46	1	Fe^{++}	2,945,000	4.8	
	1/17/44	14.1	5.84	14.4	44	1	Fe^{+++}	2,344,000	5.2	0.9

* Highest value reached.

ferric or the ferrous state. At the time of the second dose, a fairly constant level of labeled iron had been reached in the blood as a result of the first dose. The increase above this level which followed the second administration was taken to be the percentage of absorption of the second dose.

II. ABSORPTION OF FERROUS AND FERRIC IRON BY THE DOG

Six normal dogs were given 1 mgm. per kgm. of either ferrous or ferric radioactive iron and the appearance of the labeled element as newly synthesized hemoglobin in the peripheral blood was determined as a measure of the amount absorbed. After 2 to 3 weeks, each animal was fed a comparable dose of the valence form not given at the first administration, and the observations were repeated. Determinations were made on small samples of blood (5 to 10 ml.), collected at intervals of 3 days.

The results of these experiments are summarized in Table I, and representative data are charted in Figures 1 and 2. It will be observed, first of all, that the quantities of iron absorbed by these "normal" dogs were usually several times greater than the fraction of 1 per cent reported by Hahn and his associates (26, 27). There are several possible explanations for these differences. The 6 animals used may not have had adequate iron reserves although they had been given vermifuges and had been kept in individual cages on a Purina dog chow diet for weeks before being used. Their erythrocyte, hemoglobin, and hematocrit levels were well within the normal range. They were not, however, made "plethoric" by transfusion nor given iron supplements prior to the experiments. The test dose of iron, furthermore, was given in solution by stomach tube, to the fasting dog. In the studies reported by Hahn and co-workers (26, 27), the iron was mixed with food and fed, a practice which provided more favorable conditions for the formation of insoluble forms of iron in the intestinal tract (38). The differences in the absolute amount of iron absorbed, however, do not influence the following comparison between the relative absorption of the 2 valence forms. In 6 of 9 comparisons made in the 6 animals, more ferrous than ferric iron was assimilated while in

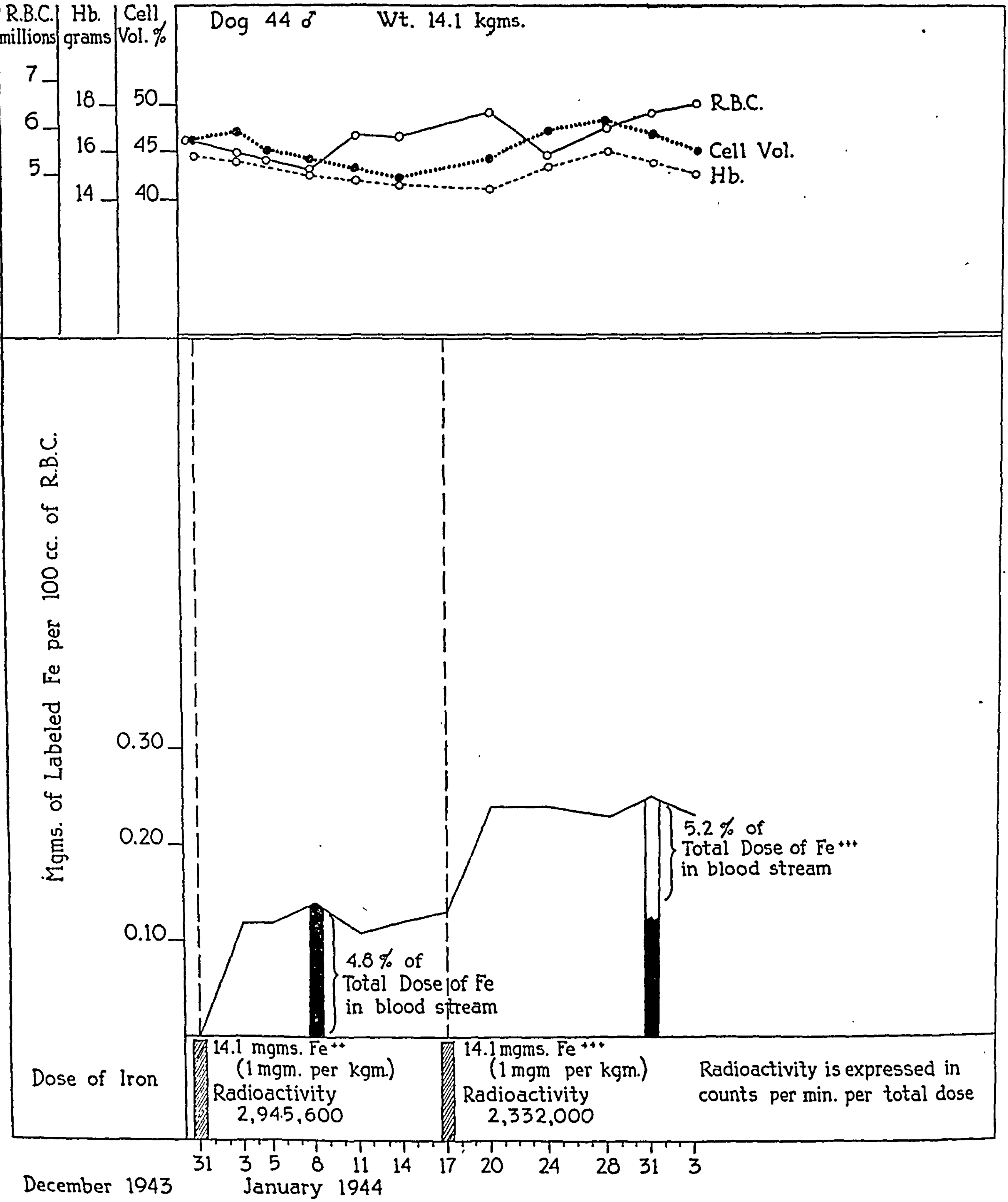


FIG. 1. ABSORPTION OF FERROUS AND FERRIC IRON BY A NORMAL DOG

In this and subsequent figures, the "labeled" or radioactive iron is charted in terms of mgm. per 100 cc. of red cells. The solid black vertical bar represents the percentage of the first test dose present in the calculated circulating blood volume. In order to estimate the percentage of iron absorbed from the second dose, it was necessary to assume that the quantity of radioactive iron present in the blood as the result of the first dose remained constant throughout the second period. The percentage absorbed from the second dose is represented by the clear portion of the second vertical bar. Any error which results because the curves are superimposed would tend to make the figure for absorption during the second period lower than it should be.

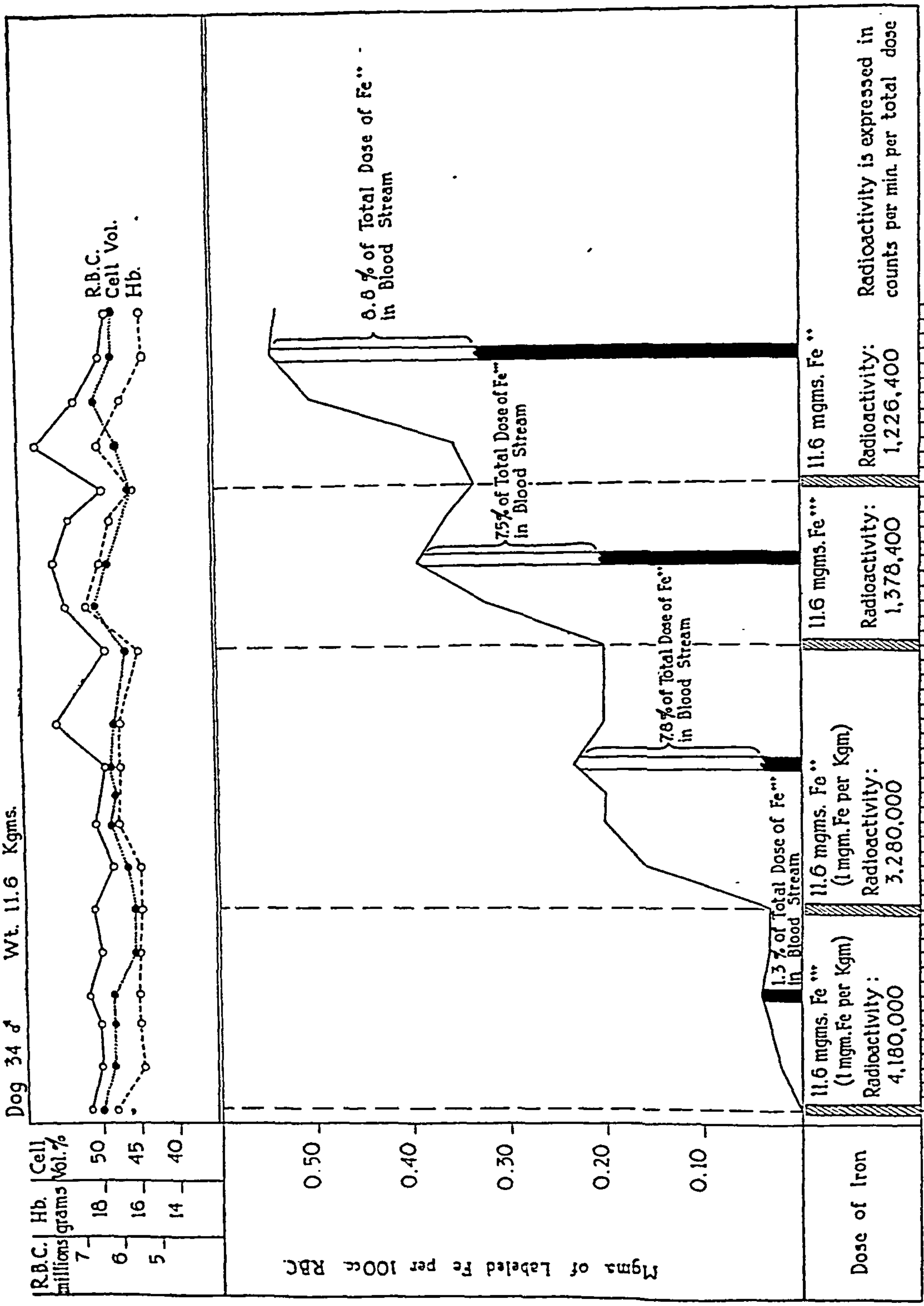


Fig. 2. Absorption of Ferrous and Ferric Iron by a Normal Dog.

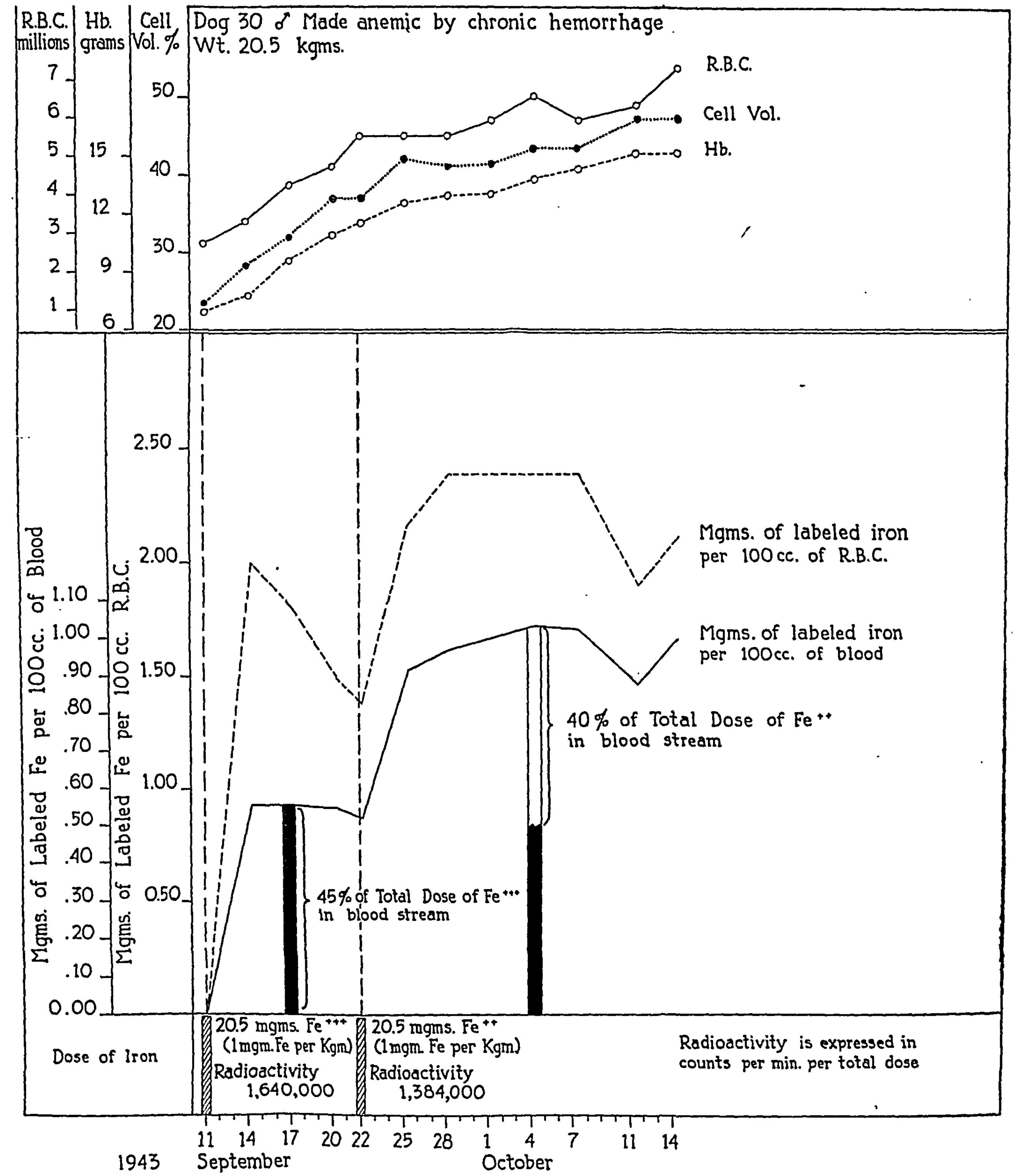


Fig. 3. Absorption of Ferrous and Ferric Iron by an Anemic Dog

the other 3 comparisons, almost equal amounts of the 2 forms were absorbed (Figure 1, Table I). Two sets of determinations were made on Dogs 32, 34, and 38. More ferrous than ferric iron was absorbed by Dogs 32 and 34 at the time of the first comparison (Figure 2), whereas in the second set of experiments, no difference existed. The explanation for the greater assimilation of ferrous salts on one occasion in these animals is not clear. Certainly the amount of blood with-

TABLE II

Absorption of ferric and ferrous iron by dogs made anemic by chronic hemorrhage

Dog	Date	Wt.	R.B.C.	Hgb.	Cell vol.	Dose of Fe	Valence of iron	Radioactivity	Labeled iron in blood stream*	Ratio of amount of Fe^{++} to Fe^{+++} absorbed
		kgm.	*millions*	*grams*	*per cent*	*mgm. per kgm.*		*counts per min. of total dose*	*per cent*	
15	4/ 7/41	13.6	4.00	7.6	25	3.6	Fe^{++}	9,540†	10.8	
	4/19/41		3.96	7.5	25	3.6	Fe^{+++}	8,400	11.3	1.0
21	6/29/43	13	2.67	4.5	17	1	Fe^{+++}	8,135,000	21.7	
	7/29/43		4.75	6.2	21	1	Fe^{++}	3,920,000	22.5	1.0
30	9/11/43	20.5	2.71	6.9	23	1	Fe^{+++}	1,640,000	45.0	
	9/22/43		5.5	11.4	37	1	Fe^{++}	1,384,000	40.4	0.9
31	8/13/43	16.8	2.75	5.6	24	1	Fe^{++}	1,952,000	29.5	
	8/25/43		6.15	12.2	43	1	Fe^{+++}	1,680,000	19.1	1.5

* Highest value reached. † Made with Bale dip type counter.

drawn for determinations was not great enough to have influenced materially their iron reserves.

Similar observations were made on 4 anemic dogs whose iron reserves had been depleted by systematic bleeding. The test dose of radioactive iron was 1 mgm. per kgm. of body weight in 3 dogs and 3.6 mgm. per kgm. in the fourth. In all instances, there was: (*1*) greater assimilation than had occurred in the "normal" dogs, and (*2*) approximately equal assimilation of the 2 valence forms (Table II, Figure 3). It will be noted, however, that Dog 31 absorbed 29 per cent of the ferrous salt and only 19 per cent of the ferric. The ferric test dose, however, was given after the animal had almost recovered from his anemia; this fact may, partially at least, have accounted for the difference.

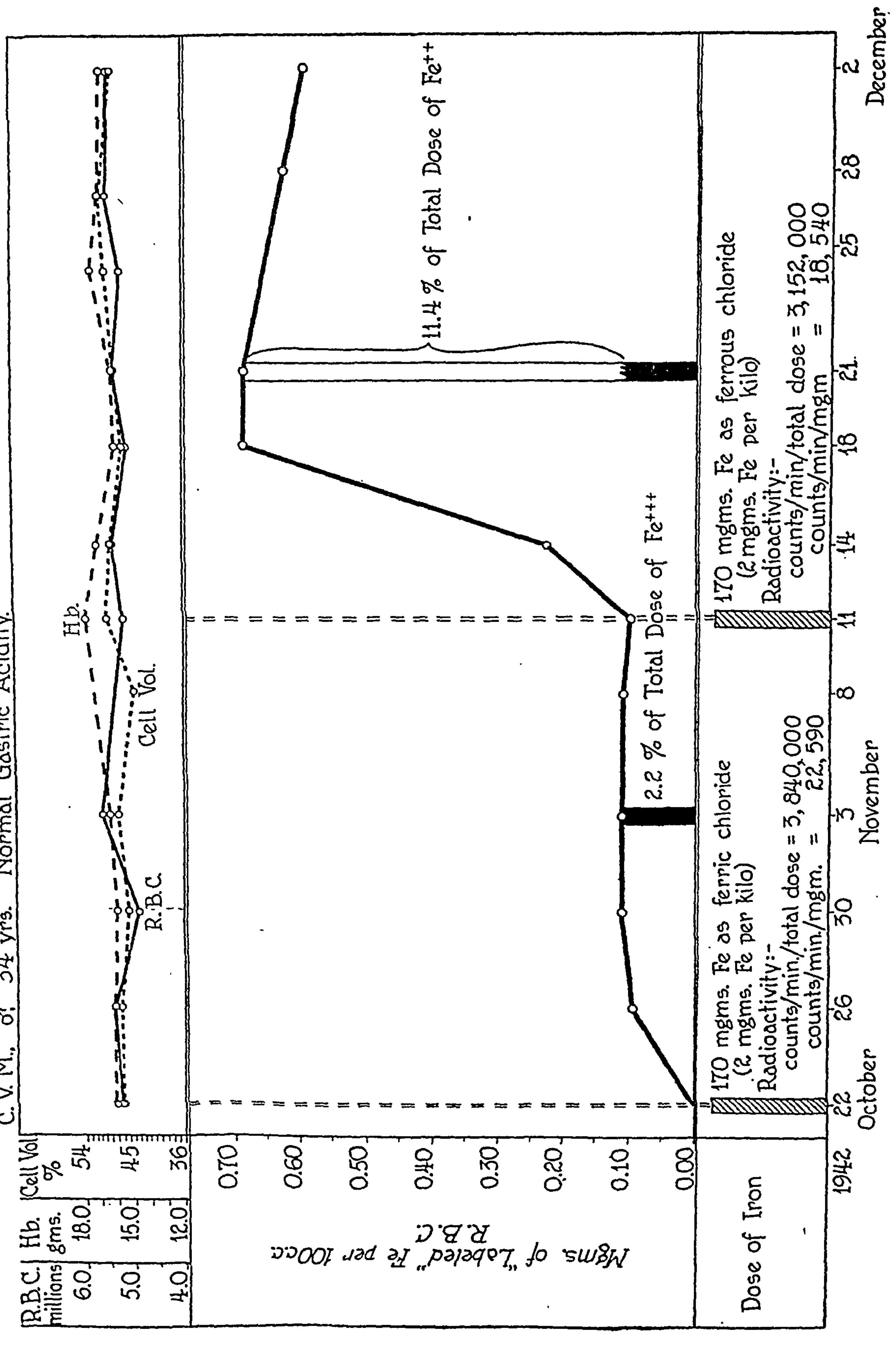
Under the conditions of these experiments,

TABLE III

Absorption of ferric and ferrous iron by healthy men

Subject, age	Date	R.B.C.	Hgb.	Cell vol.	Dose of Fe	Valence of iron	Radioactivity	Labeled iron in blood stream*	Ratio of amount of Fe^{++} to Fe^{+++} absorbed
years		*millions*	*grams*	*per cent*	*mgm. per kgm.*		*counts per min. of total dose*	*per cent*	
EHK.	4/30/42	5.37	16.2	47	4	Fe^{+++}	427,200	0.8	
23	5/18/42	4.87	15.5	46¼	4	Fe^{++}	316,000	8.0	10
JRC.	4/30/42	5.32	16.0	48	4	Fe^{++}	315,200	5.9	1½
22	5/18/42	5.05	15.9	47½	4	Fe^{+++}	233,600	3.4	
LWM.	10/22/42	4.67	14.5	43	4	Fe^{+++}	6,240,000	0.9	
28	11/14/42	4.61	14.0	43	4	Fe^{++}	4,410,000	4.6	5
CVM.	2/ 3/42	4.74	15.4	46	4	Fe^{+++}	970,000	4.4	
33	2/24/42	4.65	15.3	47	4	Fe^{++}	821,000	9.5	2
CVM.	10/21/42	5.25	16.2	47	2	Fe^{+++}	3,840,000	2.2	
34	11/11/42	5.20	17.2	49	2	Fe^{++}	3,152,000	11.2	5
JO.	5/ 1/42	4.96	16.2	47	2	Fe^{++}	213,600	10.6	
23	5/18/42	5.01	15.8	48	2	Fe^{+++}	162,400	3.5	3
TGS.	7/ 2/42	5.21	14.7	46	2	Fe^{+++}	1,080,000	0.3	
22	7/18/42	4.57	14.1	42	2	Fe^{++}	854,000	1.1	3
CCJ.	11/26/43	5.39	15.5	48½	1	Fe^{++}	9,876,000	9.3	
22	12/24/43	5.20	15.6	48½	1	Fe^{+++}	6,672,000	1.4	7
GB.	11/26/43	5.53	17.9	54	1	Fe^{+++}	9,876,000	1.3	
23	12/24/43	5.56	16.4	50	1	Fe^{++}	6,672,000	8.1	6

* Highest value reached.



Note: 20 c.c. samples of blood used for determinations.

FIG. 4. ABSORPTION OF FERRIC AND FERROUS IRON BY A NORMAL ADULT MALE

TABLE IV

Absorption of ferric and ferrous iron by subjects with hypochromic anemia

Subject, age	Date	R.B.C.	Hgb.	Cell vol.	Dose of Fe	Valence of iron	Radioactivity	Labeled iron in blood stream*	Ratio of amount of Fe^{++} to Fe^{+++} absorbed
years		*millions*	*grams*	*per cent*	*mgm. per kgm.*		*counts per min. of total dose*	*per cent*	
MBW.	10/24/42	3.65	8.7	30	4	Fe^{+++}	5,150,000	3.5	
16	11/21/42	3.78	7.6	29	4	Fe^{++}	3,564,000	11.9	3
RLB.	11/23/42	4.23	5.7	27	4	Fe^{+++}	226,000	4.3	
38	12/15/42	4.36	5.6	27	4	Fe^{++}	173,000	13.1	3
AL.	11/21/42	3.53	7.0	26	2	Fe^{+++}	254,000	1.9	
38	12/21/42	3.57	7.8	28	2	Fe^{++}	194,000	29.4	15
AR.	12/ 5/42	3.29	9.3	30	2	Fe^{+++}	6,060,000	6.2	
38	1/ 4/43	3.52	9.2	31	2	Fe^{++}	4,380,000	26.1	4
ML.	9/13/43	4.41	9.1	35	1	Fe^{+++}	4,442,500	17.0	
38	9/29/43	4.59	9.2	33	1	Fe^{++}	3,225,000	39.7	2

* Highest value reached.

therefore, the dog may either absorb both valence forms of iron equally well or show preferential assimilation of the ferrous form. It is of interest that equal absorption was obtained on all 4 of the anemic dogs but in only one-third of the experiments on normal animals. The fact that the ferrous form was more efficiently assimilated in roughly half of the experiments is compatible with the concept that the dog must reduce ingested iron to the bivalent state before absorbing it. If reduction is slow or incomplete, decreased absorption of ferric iron may result. Further work will be necessary to test the validity of this explanation.

III. ABSORPTION OF FERROUS AND FERRIC IRON BY THE HUMAN SUBJECT

Comparison of the absorption of radioactive ferrous and ferric iron was made 9 times on 8 different adult male subjects. The test doses used were 1, 2, and 4 mgm. of iron per kgm. of body weight and were always given after a 12-hour fast. Adult males were selected to avoid the complication of blood loss incident to menstruation in women. The erythrocyte, hemoglobin, and hematocrit values were normal in all instances. In each case, more ferrous than ferric iron was absorbed; the ratio Fe^{++} to Fe^{+++} assimilated varied from $1\frac{1}{2}$ to 10 (Table III, Figure 4). It is also worth noting that only 2 subjects absorbed less than 5 per cent of the ferrous test dose.

Similar studies were made on 5 patients with severe degrees of hypochromic microcytic anemia (Table IV, Figures 5 and 6). Absorption of the bivalent form was 2 to 15 times as great as that of the ferric form. Note should also be made of the fact that, as in animal experiments (26,27), the percentage assimilated was greater with the small than with the large doses. There was a distinct tendency, furthermore, for these iron-deficient subjects to assimilate iron more completely than did the normal adult males.

These results confirm, therefore, the conclusion of clinical investigators (1 to 13) that the human subject absorbs ferrous iron more efficiently than ferric.

IV. DISCUSSION

In the debate between clinical and animal investigators as to the valence form in which iron is absorbed from the intestinal tract, no single group of investigators has previously studied both human subjects and animals. The present study represents such an investigation and has used what seems to be the best method available for quantitating iron absorption. Human subjects were shown to absorb ferrous iron more efficiently than ferric iron. Dogs assimilated both valence forms equally well in 7 out of 13 comparisons; in the other 6 experiments, more ferrous than ferric iron was absorbed.

There are 3 possible explanations for the greater absorption of bivalent iron by the human: (*1*) only ferrous iron may be absorbed and all trivalent iron may have to be reduced before it

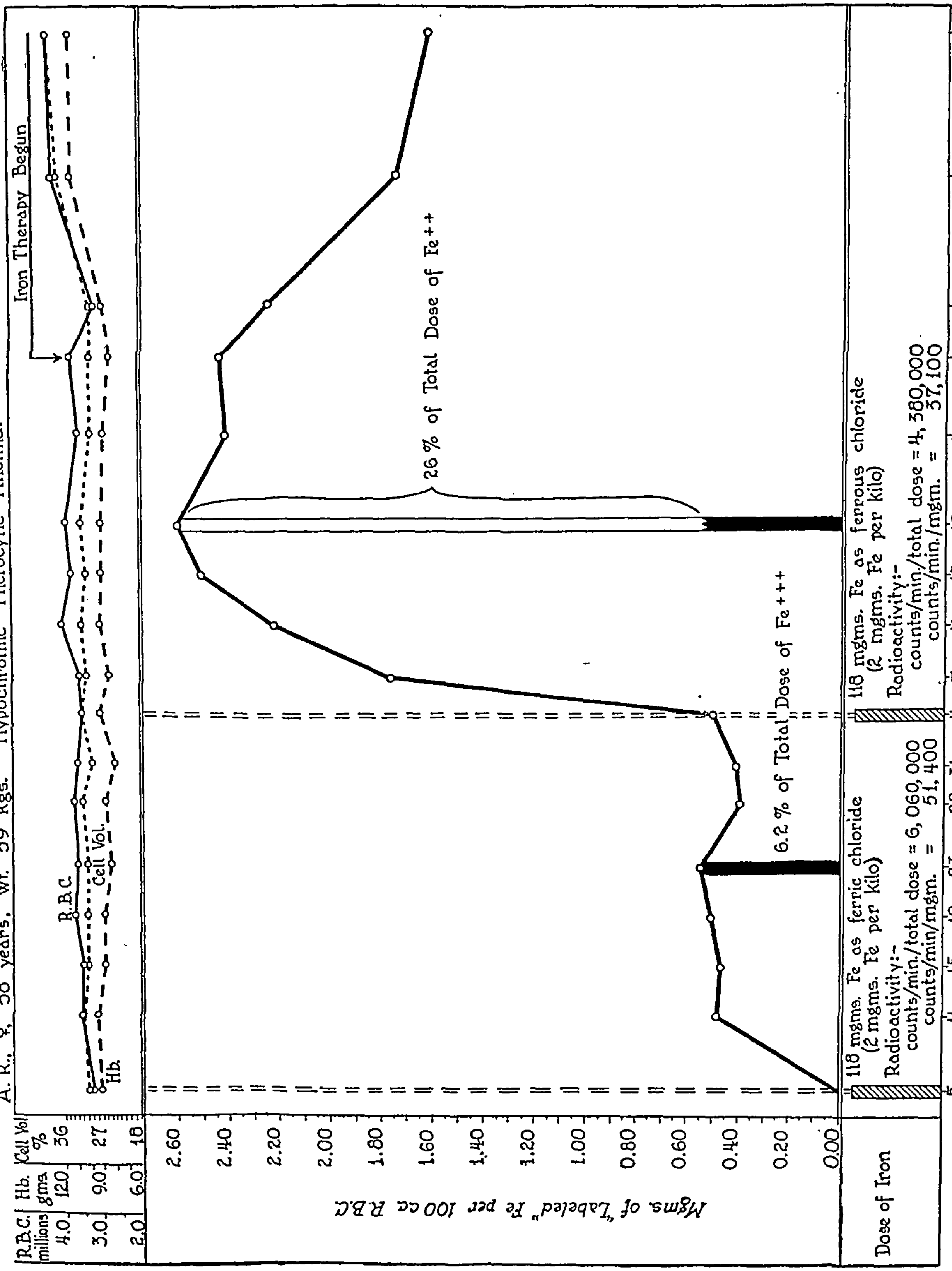


FIG. 5. ABSORPTION OF FERRIC AND FERROUS "LABELED" IRON BY A SUBJECT WITH HYPOCHROMIC ANEMIA

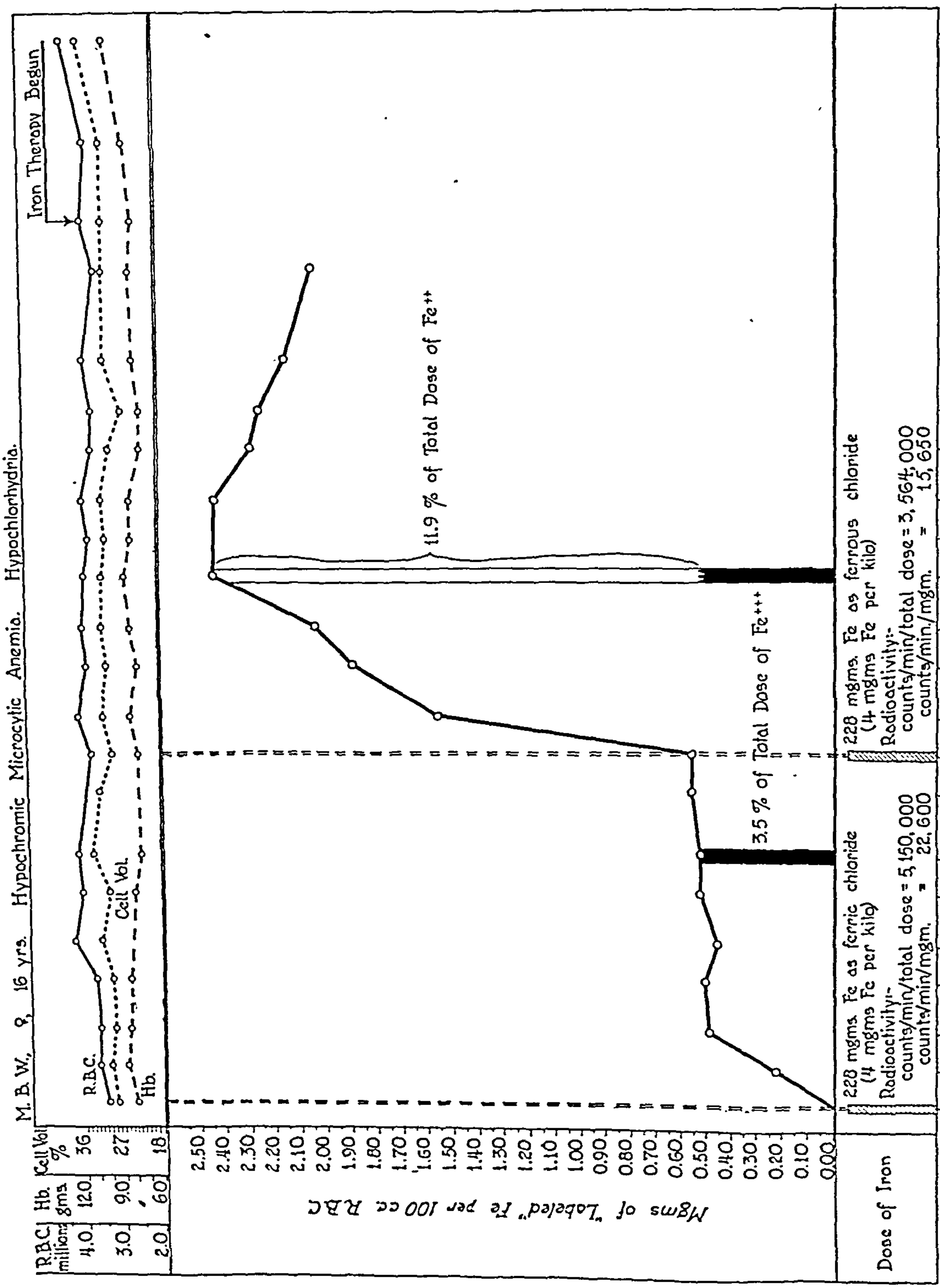


FIG. 6. ABSORPTION OF FERRIC AND FERROUS "LABELED" IRON BY A SUBJECT WITH HYPOCHROMIC ANEMIA

can be taken up by the body; (*2*) both forms may be absorbed but to an unequal degree; and (*3*) ferric iron may be made less available for absorption because it more readily forms complex insoluble compounds within the intestinal tract. How the dog differs from the human so that it frequently can absorb ferrous and ferric iron equally well can only be conjectured. The dog may reduce ferric iron more efficiently than does the human. Its intestinal mucosa may absorb both forms without distinction or conditions may be less favorable for the formation of insoluble ferric compounds. It has been reported that rats absorb radioactive ferrous and ferric iron equally well (18). Whatever the reasons, the data help provide an explanation for the different opinions expressed by human and animal investigators. When discussion is made of the valence form in which iron is most readily absorbed from the intestinal tract, the discussion must be related specifically to the species of animal under consideration.

V. SUMMARY

Comparison was made in the same "normal" and iron-deficient human subjects and in the same "normal" and iron-deficient dogs of the degree to which comparable test doses of ferrous and ferric radioactive iron were assimilated. The quantity of iron given varied from 1 to 4 mgm. of iron per kgm. of body weight and the administration was made under fasting conditions. The amount of radioactive iron which subsequently appeared as hemoglobin in the peripheral blood was used as the measure of the amount absorbed. Under these conditions, human subjects absorbed 1½ to 15 times more ferrous than ferric iron, while dogs either absorbed both valence forms to a comparable degree or showed preferential assimilation of ferrous salts. Because species of animals have been shown to differ in this respect, discussion of the valence form in which iron is most readily absorbed from the intestinal tract should be related to the species under consideration.

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THE RENAL EXCRETION OF CHLORIDE AND WATER IN DIABETES INSIPIDUS

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The primary purpose of these studies was the development of an objective test for distinguishing a sustained diuresis resulting from excessive and habitual water drinking from the polyuria caused by a defect of the neurohypophysis. It is now well established that the hypothalamico-hypophyseal mechanism contributes to control of the renal excretion of water by regulating the release of pituitrin. A technic for testing the functional integrity of this system is based on the demonstration by Hare, Hare, and Phillips (1) that the renal response to the administration of hypertonic saline is consistently different in normal dogs and in dogs with experimental diabetes insipidus. They found that the effect of pituitrin during a saline diuresis could be more clearly revealed by centering attention upon the character of the tubular reabsorbate than upon values in the urine; *i.e.*, upon the concentration of chloride in the water taken from glomerular filtrate and restored to the blood, regardless of tubular regions involved in this process. The relation of chloride concentration in the reabsorbate to that in plasma is defined by the R/P ratio. This ratio changes in the normal dog throughout the course of a saline or water diuresis. When hypotonic saline is given intravenously or water by mouth, the chloride R/P rises rapidly from its basal value of one; but when strongly hypertonic saline is injected, the chloride R/P falls progressively. In diabetes insipidus, however, the chloride R/P is initially greater than one and during the infusion of hypertonic saline, it usually increases. The addition of pituitrin to the infusion fluid restores the response to normal, but the quantity of hormone required (500 to 1000 milliunits) is at least 100 times that liberated within the same period from the pituitary of a normal dog under basal conditions (2). It is concluded that, in the normal dog, hypertonic saline causes the release of large amounts of pituitrin from the neurohypophysis.

If the neurohypophyseal mechanism in man responds in the same manner, its functional capacity should be evident in the renal response of a patient to the intravenous infusion of hypertonic saline. The observations on patients were made by applying the same procedures used by Hare, Hare, and Phillips (1) with 2 modifications: glomerular filtration, necessary in the calculation of tubular reabsorption, was measured by inulin instead of creatinine clearances, and the quantity of infusion fluid, expressed in relation to body weight, was reduced by more than half. Of secondary interest was the comparison of the renal excretion of salt by normal subjects and by patients with diabetes insipidus. White and Findley (3) reported that salt given by mouth was excreted more slowly than normal by patients with diabetes insipidus, but that pituitrin was not effective in correcting this fault. We (1) failed to find any difference in the renal excretion of salt injected intravenously into normal dogs and those with experimental diabetes insipidus, but since the species and the route of administering the salt were not the same, we extended our observations to man and gave the test dose of salt by mouth and by injection.

PROCEDURES

Seven consecutive clearance periods of about 15 minutes each were run on all patients. The first 2 periods served as controls; during the next 3, a saline solution was infused intravenously at 0.25 cc. per kgm. per minute. Twenty cc. of a 10 per cent inulin solution in isotonic saline were given intravenously 15 minutes before the beginning of the first period and continued as a sustaining infusion at 0.5 cc. per minute throughout the experiment. Urines were collected by catheter and complete emptying of the bladder was assured by repeatedly washing the bladder with air at the end of each clearance period. Blood samples were collected before the experiment (for the inulin blank) and at the midpoint of each period. They were drawn under oil without an anticoagulant and centrifuged immediately.

Thirteen experiments of the type described above were done on 5 normal subjects and 3 patients who were routinely taking pitressin tannate in oil to control their urine

volume. For convenience, the term "polyuric" is used to designate these patients, without regard for the cause of their increased water exchange. Pitressin therapy was discontinued and the urine flow allowed to reach a plateau in the patients before any observations were made. In the studies on the renal excretion of chloride, the data from these infusion experiments were used in addition to those obtained on 3 polyuric and 2 normal subjects given salt by mouth. At first, these latter experiments were done according to the schedule of White and Findley (3), but later were extended to cover 24-hour water and chloride exchange. Under this regimen, the urines were voided and pooled into 12-hour samples.

Chlorides in urine and serum were determined by the silver nitrate method of Van Slyke and McLean (4) or by the mercurometric titration of Schales and Schales (5). After glucose was removed by fermentation, inulin analyses were done according to Harrison's (6) modification of the diphenylamine method with a Klett-Summerson colorimeter.

CLINICAL MATERIAL

The case histories of the 3 polyuric patients who received the intravenous saline injections are given below.

L. S. No. 42–5976. This 16-year-old male has had a polyuria for 12 years. He was a premature baby with a birth weight of 1.7 kgm. and developed poorly. At 4 years of age, he began to excrete large volumes of urine (almost 6 liters daily) of low specific gravity. Pituitrin nose-drops were used at first to control the polyuria, but were later replaced with pitressin tannate in oil with great success. The urine has been sugar-free on repeated tests and the glucose tolerance test normal. Without pitressin therapy, the daily urine volume is now 8 to 10 liters with a specific gravity of 1.001 to 1.003. X-ray films of the head show no abnormality. Growth has been subnormal throughout life; at the age of 4, he weighed 12.5 kgm. and was 36 inches high; at 15, he weighed 31 kgm. with a height of 55 inches and a skeletal age of 13 according to Todd's Atlas. At the time of this admission, he weighed 40.5 kgm.

M. McC. No. 39–387. This patient is a 20-year-old male who has Schüller-Christian's syndrome with diabetes insipidus. He has been admitted to the hospital 8 times within the past 5 years. The first admission at the age of 15 was because of a severe weight loss, and on the pediatric service, it was noted that he excreted more urine than normal and that it was of low specific gravity. This polyuria increased in severity and when the patient was 17 he was placed on a regimen of nasal insufflations of posterior pituitary powder. Without therapy, the daily urine volume was 12 to 15 liters. In May, 1940, a diagnosis of Schüller-Christian's disease was made and a lipoid granuloma removed from the right mastoid process. His teeth began to fall out in 1938 and in 1941 complete dentures were obtained. X-ray plates of the head showed no lesions in the region of the pituitary fossa. The patient weighed 52 kgm. at the time these experiments were performed. One cc. of pitressin tannate in oil controlled the polyuria for 5 to 7 days.

M. M. No. 40–2895. This patient is a 47-year-old white housewife who has been receiving pituitrin or pitressin tannate in oil for the past 10 years to control a polyuria and polydipsia which began suddenly in 1933 without any history of preceding illness or trauma. Two months after the onset of her polyuria, her visual fields and the x-ray plates of her head were normal. The Wasserman was negative. The daily urine volume varied from 4 to 9 liters, and when water was withheld, the patient became weak and ill. At this time, the patient was given 1 cc. of pituitrin 3 times daily, but for economic reasons, posterior pituitary powder by nasal insufflation was substituted. A week after discharge from the hospital, the patient returned to pituitrin injections. In March, 1940, the patient was put on pitressin tannate in oil, receiving an injection every 12 hours. Her weight on admission, April 16, 1943, was 78 kgm.

The normal subjects were free from renal disease and in good health.

RESULTS

Since these observations are an extension of those made on dogs, the first 3 experiments were done on a normal subject to establish that the human kidney responded like that of the dog to saline solutions of different concentrations. The data from 1 of these experiments, in which 2.5 per cent NaCl was injected, are given in detail in Table I. The temporal course of the chloride R/P of the 3 experiments, graphically shown in Figure 1, is similar to that reported for dogs (1). The elevation of the chloride R/P during the infusion of hypotonic salt solution is attributed

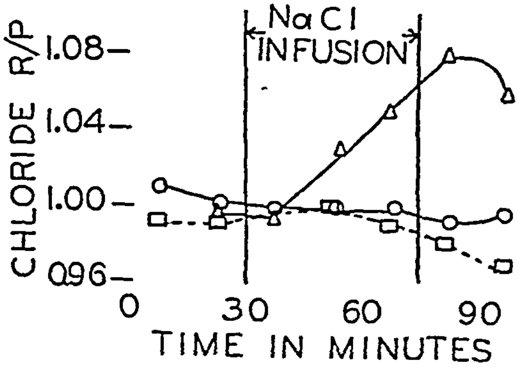

FIG. 1. THE TEMPORAL COURSE OF THE CHLORIDE R/P IN A NORMAL SUBJECT

Receiving 0.6 per cent (triangles), 0.9 per cent (circles), and 2.5 per cent (squares) NaCl solution intravenously at the rate of 0.25 cc. per kgm. per minute.

TABLE I

The effects of intravenous injection of 2.5 per cent sodium chloride into normal and polyuric patients

Subject and period		Duration	Urine flow	Inulin clearance	Inulin U/P	Plasma chloride	Chloride filtered	Chloride excreted	Chloride reabsorbed	Water reabsorbed	Chloride in tubular reabsorbate	Chloride R/P	Urine chloride
		minutes	*cc. per minute*	*cc. per minute*		*mgm. per cent*	*mgm. per minute*	*mgm. per minute*	*mgm. per minute*	*cc. per minute*	*mgm. per cent*		*mgm. per cent*
Normal	1	16.8	2.62	173	66	381	659	13.9	645	171	377	0.99	532
K. H.	2	14.0	2.34	182	78	380	691	12.8	678	180	377	0.99	547
	3	16.4	4.28	215	51	385	830	21.0	809	211	383	1.00	491
2.5 per cent sodium	4	15.3	6.23	200	32	401	802	28.4	774	194	399	1.00	456
chloride	5	12.5	5.62	191	34	412	788	34.9	753	185	407	0.99	622
	6	14.5	4.65	192	41	410	787	33.7	753	187	402	0.98	725
Weight, 72 kgm.	7	15.5	4.28	187	44	415	776	34.9	741	183	405	0.97	816
Diabetes insipidus	1	14.3	7.18	53	7.4	397	210	4.7	205	46	445	1.12	65
L. S.	2	13.7	6.52	53	8.1	400	212	4.7	207	46	450	1.12	72
	3	16.3	8.53	56	6.5	404	226	7.9	218	47	464	1.15	93
2.5 per cent sodium	4	17.1	10.41	62	5.9	415	257	13.6	243	52	468	1.13	131
chloride	5	12.5	11.90	64	5.4	419	267	17.9	249	52	479	1.14	150
	6	12.9	11.30	62	5.5	421	261	19.5	241	51	472	1.12	173
Weight, 40 kgm.													
Diabetes insipidus	1	16.8	5.28	59	11	376	222	1.1	221	54	409	1.08	21
M. McC.	2	14.1	5.73	59	10	385	227	1.7	225	53	425	1.10	30
	3	15.3	6.88	59	8.6	400	236	2.5	233	52	448	1.12	36
2.5 per cent sodium	4	14.6	7.52	65	8.6	415	270	2.6	267	57	468	1.13	35
chloride	5	16.5	7.82	61	7.8	410	250	2.5	247	53	466	1.14	32
Weight, 52 kgm.													

to a diminution of the amount of pituitrin in the blood; the unchanged chloride R/P with isotonic saline indicates little change, if any, in the activity of the neurohypophysis; and the fall of the chloride R/P when hypertonic saline was given signifies the release of pituitrin. In contrast to this, when 2.5 per cent NaCl solution was given to 2 patients (L. S. and M. McC., Table I) with diabetes insipidus, the chloride R/P, already greatly elevated, increased further (Figure 2). The injection of 0.02 cc. (400 milliunits) of pituitrin along with the saline into 1 of these patients (L. S.) sharply depressed the chloride R/P (Figure 2) and the renal response simulated that of the normal subject.

The responses of the third polyuric patient (M. M.) were normal (Figure 2) and in sharp contrast to those of the other 2. Three infusions of 0.9 per cent, 2.5 per cent, and 5 per cent NaCl were given and, in all experiments, the results indicate a normal control of the excretion of urine. Not only did the response of the patient's kidneys indicate the presence of an antidiuretic substance, but the excretion of the material in her urine was demonstrated. Thirty-minute urine samples were collected immediately before and after the infusion of 250 cc. of 5 per cent NaCl solution and half of each injected intravenously into a dog with diabetes insipidus according to the method of Hare, Hickey, and Hare (7). Each injection was followed by a period of diminished urine flow which was not caused by a reduction of glomerular filtration, but by an increase in the tubular reabsorption of water (Figure 3). The preliminary injection of 1 milliunit of pituitrin was intended to establish the sensitivity of the test animal to the antidiuretic hormone and to provide a response to which subsequent responses could be compared. When the basal urine flow of the test animal remains constant, a high correlation exists between the response and the dose of pituitrin (8), but when the basal flow changes, as in this assay, the responses to successive doses cannot be accurately compared. However, the presence of an antidiuretic substance in the urine of M. M. is clearly shown, and, on repetition of the assay on another dog, results practically identical with those shown in Figure 3 were obtained. The significance of the antidiuretic action of this

patient's urine is enhanced by the observation that urines from 3 other untreated polyuric patients had a diuretic effect when similarly tested. When pituitrin was injected into 1 of these patients, an antidiuretic substance immediately appeared in the urine. This is in exact agreement with the findings of Hare, Hickey, and Hare (7) that the urine from untreated dogs with experimental diabetes insipidus augmented the urine flow of the test animal, while urine collected from the same dogs after the injection of pituitrin produced an antidiuresis.

These results are so nearly identical with those obtained in dogs, both normal and with experimental diabetes insipidus, that the test for neurohypophyseal function in man is considered valid. However, its complexity makes its clinical application difficult, and a simpler indicator of the presence or absence of pituitrin is the concentration ratio of chloride (chloride U/P) which can be determined without measuring glomerular filtration. The effect of pituitrin, as indicated by the chloride U/P, is illustrated in Figure 4. The low chloride U/P ratios of the 2 patients with diabetes insipidus are contrasted with the sharp elevation that occurs when pituitrin is added to the saline. To show that the infusion of hypertonic saline is effective in releasing pituitrin during a water diuresis, a normal subject was given 20 cc. of water per kgm. of body weight during the hour that preceded the infusion of saline. The increase of the chloride U/P (Figure 4) indicates that the hypertonic salt liberates pituitrin in the hydrated as well as in the untreated normal subject.

Another indicator is urine flow, and since it is, of course, easily measured, its use offers a great advantage. Since a very small dose of pituitrin will sharply depress a water diuresis in a normal subject (9), the intravenous injection of hypertonic saline should also interrupt the course of the diuresis, by causing a release of pituitrin from

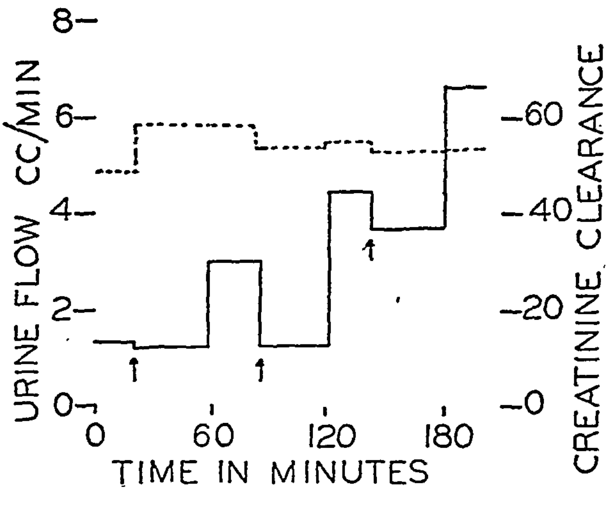

FIG. 3. DEMONSTRATION OF AN ANTIDIURETIC MATERIAL IN THE URINE OF M. M. BEFORE AND AFTER THE INJECTION OF 250 CC. OF 5 PER CENT NACL SOLUTION

The solid line indicates the urine flow of the test animal, a dog with diabetes insipidus; the broken line, the rate of glomerular filtration in cc. per minute as determined by creatinine clearances. At the first arrow, 1.0 milliunit of pituitrin was injected; at the second arrow, 45 cc. (15-minute specimen) of urine collected immediately before; and at the third arrow, 22 cc. of urine (15-minute specimen) collected from M. M. immediately after the salt injection.

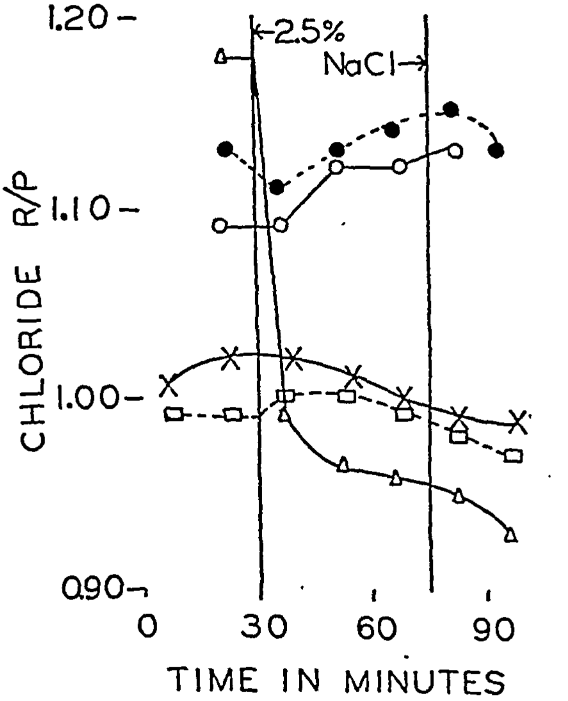

FIG. 2. THE TEMPORAL COURSE OF THE CHLORIDE R/P WHEN 2.5 PER CENT NACL IS INFUSED AT THE RATE OF 0.25 CC. PER KGM. PER MINUTE INTO POLYURIC PATIENTS (SOLID CIRCLES, L. S.; OPEN CIRCLES, M. McC.; CROSSES, M. M.) AND INTO A NORMAL (SQUARES)

The addition of 400 milliunits of pituitrin to the infusion fluid changed the response (triangles) of L. S. from that of a patient with diabetes insipidus (solid circles) to one comparable with the normal (squares).

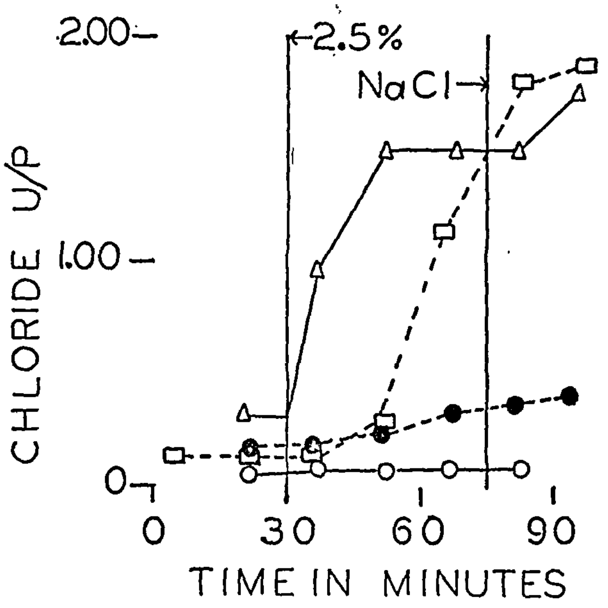

FIG. 4. THE CHLORIDE U/P OF 2 UNTREATED POLYURIC PATIENTS, L. S. (SOLID CIRCLES) AND M. McC. (OPEN CIRCLES), OF A HYDRATED NORMAL SUBJECT (SQUARES), AND OF A POLYURIC PATIENT, L. S., RECEIVING PITUITRIN (TRIANGLES) BEFORE, DURING, AND AFTER THE INTRAVENOUS INFUSION OF HYPERTONIC SALT SOLUTION

the subject's neurohypophysis. This is illustrated in Figure 5. Minute amounts of pituitrin also control the polyuria of a patient with a lesion of the hypothalamico-hypophyseal system, but the injection of hypertonic saline, instead of inhibiting the polyuria, causes a further and striking increase in the urine flow. The greatest difference in the responses is seen in the first period after the infusion is discontinued when the urine flow of the patients with diabetes insipidus is 6 and 9 times as great as that of the normal subject. The use of urine flow as an indicator requires the preliminary hydration of the subject, as an antidiuresis cannot be evaluated if the patient is already oliguric. As a matter of fact, under these circumstances hypertonic saline may be diuretic (See experiment 1, Table I).

When the quantity of chloride excreted after an oral dose was considered, no consistent difference in the response of the 2 groups, normal and polyuric, could be detected, even when the study was followed more than 24 hours after the test dose. Once when a normal patient and one with diabetes insipidus were put on the same high salt diet, the serum chloride of the polyuric patient increased more than the normal and the urinary excretion remained lower. However, this isolated instance of chloride retention after the ingestion of salt can hardly be considered characteristic of diabetes insipidus since in 7 other experiments, the polyuric and normal subjects excreted the salt at almost identical rates.

Better information for comparing renal excretion of salt is included in the data from the experiments in which the salt was injected intravenously. Four representative cases are shown in Figure 6, in which the chloride excretion, expressed in terms of body weight, is related to time. One of the patients with diabetes insipidus (M. McC.) did excrete chloride at a subnormal rate, while the other (L. S.) responded like the normal subject. The experiment on L. S. was repeated with the addition of pituitrin, and the chloride excretion, which was higher in the control period, continued higher throughout, although the salt injections were identical. The variables in intestinal absorption which may occur after the ingestion of salt are eliminated by injecting the test dose of salt, but even under these conditions, a subnormal excretion of chloride is not a consistent finding in diabetes insipidus.

DISCUSSION

The idea that the administration of hypertonic saline causes a release of antidiuretic hormone

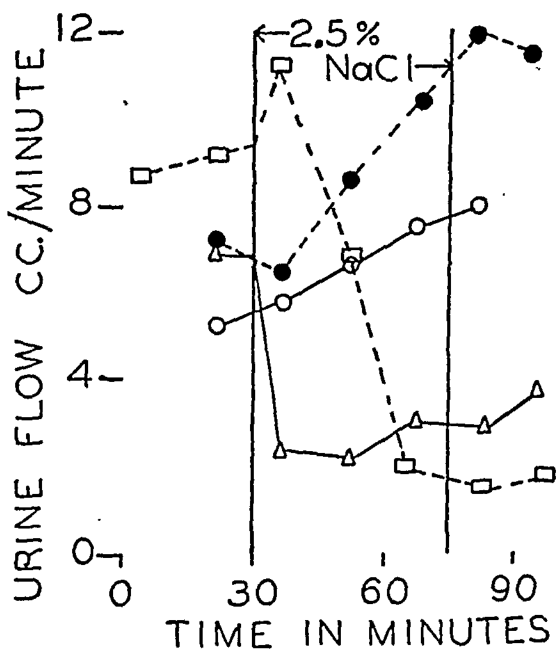

FIG. 5. URINE FLOW OF SUBJECTS RECEIVING HYPERTONIC SALINE INJECTIONS
Experiments and symbols the same as in Fig. 4.

from the pituitary seems to have originated with Gilman and Goodman (10) who used this stimulus as an alternative to withholding fluids to create a need for the conservation of water. While the 2 stimuli may act through different mechanisms, both are effective in causing the renal excretion of an antidiuretic substance. Therefore, experiments using either hypertonic saline or dehydration or both will be cited in arraying the evidence that the antidiuretic substance is of pituitary origin. In the experiments of Gilman and Goodman (10), a quantity of 5 per cent NaCl solution, equal to 5 per cent of the body weight, was given to rats and the urine collected for several hours. The antidiuretic content of the urine, estimated from its inhibitory effect on a water diuresis, was always increased following salt administration to normal animals but was entirely lacking when hypophysectomized rats were similarly treated. Dehydration produced similar results, which have been confirmed, so far as the normal rats are concerned, by Boylston and Ivy (11), and in normal cats by Martin, Herrlich, and Fazekas (12). Ingram, Ladd, and Benbow (13) combined dehydration and intravenous injection of 10 per cent saline to stimulate the renal excretion of an antidiuretic substance in normal cats and obtained results in complete agreement with those of Gilman and Goodman (10). Ingram, Ladd, and Benbow used the less drastic treatment of dehydration alone on cats with experimental diabetes insipidus, but even fatal loss of water by these animals was not attended by the excretion of a detectable amount of antidiuretic material. Walker (14) has strongly protested the pituitary origin of this antidiuretic material since he found it in undiminished amounts in hypophysectomized animals. His argument seems less forceful when one considers that his hypophysectomized animals did not have a polyuria and probably retained a remnant of neurohypophysis, for Heinbecker and White (15) have shown that when all the gland is excised a permanent diabetes insipidus is established.

In all the foregoing experiments, the antidiuretic material was recovered by dialysing the urine through collodion membranes. In order to avoid this complication, Hare, Hickey, and Hare (7) collected the urine by catheter and im-

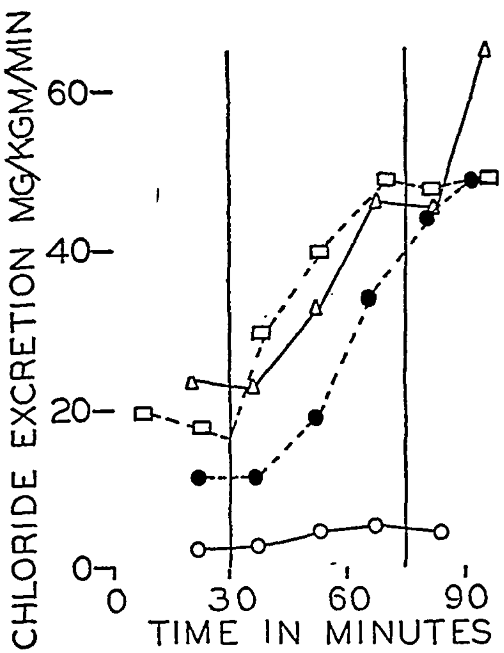

FIG. 6. THE RENAL EXCRETION OF CHLORIDE BY NORMAL AND POLYURIC PATIENTS, WITH AND WITHOUT PITUITRIN

Untreated polyuric patients, L. S. (solid circles) and M. McC. (open circles); polyuric patients, L. S., receiving pituitrin (triangles); normal subject, K. H. (squares). The vertical lines indicate the beginning and end of the infusion of 2.5 per cent NaCl at 0.25 cc. per kgm. per minute.

mediately injected it intravenously into dogs with diabetes insipidus, that developed an antidiuresis in response to as little as 0.1 milliunit of pituitrin. By this method, it has been found that withholding drinking water (7) or the injection of hypertonic NaCl or Na_2SO_4 solutions (16) caused normal dogs to excrete an antidiuretic substance in the urine. Dehydration of dogs with diabetes insipidus has no such effect, and the absence of the antidiuretic substance from the urine was correlated with its absence from the pituitary by killing the donor and assaying the atrophic pars nervosa of the pituitary and its attachment to the hypothalamus (7).

The validity of the test proposed in this paper is only partly dependent upon the pituitary origin of the urinary antidiuretic substance. It is mainly supported by the fact that the difference in the renal response to the infusion of hypertonic saline into normal dogs and into dogs without a neurohypophysis is abolished by large doses of

pituitrin (1). Whether part of the pituitrin liberated by the neurohypophysis is excreted in the urine or not, is not essential to the thesis that hypertonic saline is an effective agent for releasing the hormone.

These limited observations on patients, considered alone, scarcely provide the basis for a differential diagnostic test for diabetes insipidus of pituitary origin, but since they confirm findings on normal and polyuric dogs, on which more than 100 similar experiments have been performed, the evidence for the validity of the test is substantial. The one polyuric patient who gave evidence by this test of having an adequate endogenous source of antidiuretic material differed from the others in another respect. While she had the mildest polyuria, she received 8 to 14 times as much pitressin tannate in oil as either of the others. After it was demonstrated that she excreted an antidiuretic substance in her urine and that her responses to infusions of hypertonic saline were normal, she was persuaded to discontinue pitressin therapy for an observation period of 10 days. She developed no polyuria in that time. Three months after leaving the hospital, she reports that her water exchange has remained normal although she has received no treatment since her discharge. It is believed that her polyuria did not originate in a pituitary lesion but was the result of a polydipsia.

In the performance of the test, the subject, whether normal or polyuric, should be given 20 cc. of water per kgm. of body weight during the hour preceding the infusion of 2.5 per cent NaCl at 0.25 cc. per kgm. per minute for a 45-minute period. Measured collections at 15-minute intervals, preferably by catheter, before, during, and after the salt injection, provide all the necessary information for a differential diagnosis between a polyuria of pituitary origin and the diuresis that results from ingestion of large amounts of water. It is essential that the diuresis be well established, and that the water drinking be continued until the saline injection is begun, to be certain that any decrease in urine flow is not merely the normal decline of the water diuresis. A marked antidiuresis under these conditions indicates the release of pituitrin. This is very similar to the procedure of Gilman and Goodman and differs only in that instead of collecting that portion of the antidiuretic substance excreted in the urine and demonstrating its potency by injecting it into a second animal with a water diuresis, the antidiuretic substance liberated into the blood of the subject is detected by its direct action on his own kidneys to suppress a water diuresis.

The difference in the response of patients with diabetes insipidus and normal subjects, as measured by the changes in the chloride R/P, in the chloride U/P, or in the rate of urine flow, is abolished by replacement therapy with pituitrin. Since the differences in the renal excretion of chloride, described by White and Findley (3), and confirmed by us in 2 of 4 cases, are not corrected by pituitrin, it is improbable that a subnormal rate of urinary excretion of chloride is the fundamental fault in diabetes insipidus. The absence of pituitrin from the circulation seems to be the primary deficiency and the cause of its absence, whether hydration by water drinking or a lesion affecting the neurohypophysis, can be determined by the injection of hypertonic saline.

SUMMARY

The liberation of the antidiuretic hormone from the neurohypophysis in response to the injection of hypertonic NaCl provides a means for testing the functional capacity of the gland. The inhibition by salt injection of a pre-existing water diuresis is good evidence that the hypothalamico-hypophyseal system is in good order; a continuation or an increase of the high rate of urine flow suggests that the polyuria is of pituitary origin.

The authors take this opportunity for expressing their thanks to the Departments of Pediatrics and Medicine, for without their cooperation, these experiments could not have been performed.

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THE EFFECT OF DIODRAST ON THE NORMAL URIC ACID CLEARANCE [1]

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Uric acid clearances in man have been determined by several investigators. The values reported have ranged from 6.9 to 31.9 cc. per minute. This highest value was recently reported by one group from this laboratory (1). Subsequent attempts by us to confirm this figure have been unsuccessful. The values now obtained for uric acid clearances average about 15 cc. per minute or about one-half the value previously reported.

Many possible explanations for this discrepancy presented themselves. First, a different method is now being used for the determination of uric acid. However, the methods have been carefully checked. They give nearly identical results. And second, the uric acid clearances performed previously in this laboratory were determined upon specimens of urine and blood, obtained from patients who were also receiving inulin and diodrast, while in the later studies, inulin and diodrast were absent. The presence of either one of these substances might, therefore, alter the normal excretion of uric acid and/or affect the determination of uric acid.

It was readily determined that inulin and diodrast do not interfere with the determination of uric acid by the methods used in these experiments when these substances are added directly to the urine in quantities such that the final concentration is of the same order of magnitude as that found in the urine in clearance studies.

It would also appear that the presence of inulin in the subject does not affect the uric acid clearance since Coombs *et al.* (2) have observed an average uric acid clearance in 8 normal non-pregnant humans of 11.1 cc. per minute per 1.73 sq. m. when inulin was given simultaneously.

These observations tend to indicate that the high values for the uric acid clearances previously reported from this laboratory might be due to the simultaneous presence and excretion of diodrast. The following experiments were therefore carried out to test this hypothesis. First, we determined the effect of urine flow upon the uric acid clearance using our methods, since it is easier to obtain satisfactory urine collections for clearance determinations in the postpartum female if the urine flow is high. These experiments also assured us that we could measure the uric acid satisfactorily in very dilute urines. We then studied the effect of the injection of large quantities of glucose upon the uric acid clearance in order to determine if the reabsorption of supranormal amounts of glucose by the kidney tubule has any effect upon the uric acid clearance. We finally studied the effect of diodrast upon the uric acid clearance. Preliminary studies showed that diodrast did affect the excretion of uric acid. This effect was therefore studied in a separate series of cases.

SUBJECTS AND METHODS

Clinically normal women in their 1st to 8th postpartum day were used as experimental subjects. In the series of patients used to study the effect of water diuresis and glucose upon the uric acid clearance, sufficient water was given to insure an adequate flow. However, we also attempted to get a distribution of flows. In this series, the first clearance period was started about 9 a.m. In most cases (*i.e.* when the flow was high), the patients were not catheterized. Each clearance period averaged about 45 minutes. There were either 2 or 3 consecutive periods on the same day for each patient. Blood was drawn, either in the middle of the 2nd if there were 3 periods, or at the end of the 1st if there were 2 periods.

In the series of patients used for the diodrast study, the conditions were more rigidly controlled. At about 6 a.m., on their 4th to 6th postpartum day, the patients were given sufficient water over the period of an hour to insure an adequate urine flow. Breakfast was withheld. At approximately 7 a.m., the patient was catheterized and the urine specimen was discarded. The urine was then collected for 2 consecutive one-half hour periods. A blood

[1] This study was aided by a grant from the John and Mary R. Markle Foundation.

[2] Now, Lieutenant L. V. Dill, U. S. N.

specimen was taken after the first half-hour period. The uric acid and urea clearances were determined upon these specimens. From these data, the control clearances were calculated. On the following day, the same procedure was carried out. In addition, however, each patient was given an intravenous injection of 5 cc. of 35 per cent diodrast [3] at the beginning of each half-hour period. Diodrast was not determined. It has been assumed that the greater part of the diodrast given would be excreted in one-half hour (3) and that the blood levels of diodrast at this point would be low. The urine specimens were also analyzed for creatinine to be sure that the collection of the urine had been satisfactory. In this series, only those data have been used which showed satisfactory checks with respect to uric acid and urea clearances and creatinine excretion.

All clearances were calculated as maximum clearances, except in one instance (a urea clearance) which is indicated in the last table. Plasma values are used for the calculation of uric acid clearances. Whole blood urea values are used for the calculation of urea clearances.

Plasma uric acid was determined on Wu filtrates (4) by the method described below. Urine uric acid was determined on diluted urine by the same method. Urea in the blood and urine was determined by the manometric hypobromite method of Van Slyke and Kugel (5). Creatinine in the urine was determined by the Folin method (6). A Klett-Summerson photoelectric colorimeter (7) was used for the determination of all color densities.

RESULTS

Uric acid methods. Before these particular experiments were started, it had been found that the method previously used for the determination of uric acid (the Folin 1933 method (8) using the Folin 1934 reagent (9)) was not entirely satisfactory in our hands. The Folin 1922 method (10) which has been used for the determination of uric acid in the routine laboratory of this clinic for many years was substituted. This determination is carried out by essentially the same procedure described by Folin in 1922 except that the Folin-Marenzi reagent (11) is used and the lithium sulfate is omitted. This method will be referred to as the 1922 method.

When the discrepancy in the uric acid clearances became apparent, it became necessary to determine whether or not the 2 methods were measuring essentially the same thing. They were, therefore, compared. Simultaneous determinations by the 2 methods on the same filtrate of the uric acid content of 37 different Folin-Wu filtrates of whole blood showed that the results obtained with the 1922 method averaged 0.5 mgm. per cent lower than those obtained with the 1933 method. The whole blood uric acid values ranged from 1.8 to 5.4 mgm. per cent (1922 method). Uric acid was also estimated in the same plasma filtrates by the 2 methods. On 17 different Wu plasma filtrates, the results obtained with the 1922 method averaged 0.2 mgm. per cent lower than those obtained with the 1933 method. A similar study with several different urines at various dilutions showed that the uric acid values obtained by the 2 methods were of the same order of magnitude (Table I).

TABLE I

Urine uric acid as determined by the Folin 1922 and the Folin 1933 methods

Urine no.	Urine dilution	Uric acid	
		1922 method	1933 method
		mgm. per cent	
1	1:200	55.5	57.8
	1:100	52.7	56.9
2	1:200	32.4	37.8
	1:100	*30.3*	*30.8*
3	1:50	11.6	11.3
4	1:50	13.8	14.4
5	1:50	15.7	14.6

When inulin and diodrast are added to the urines or blood filtrates in the concentrations in which they are present in these studies, they have no effect upon the determination of uric acid. It seems, therefore, that inulin and diodrast interference with the determination of uric acid cannot account for the observed results.

There is some evidence which indicates that these methods are measuring uric acid (urate). Schaffer (12, 13) has shown that the substance in blood filtrates which produces 86 to 96 per cent of the color and the substance in urine which produces 94 to 98 per cent of the color, when the Folin 1933 method is used for the determination of uric acid, is destroyed when portions of such blood filtrates and urines are incubated with a defatted, desiccated kidney preparation which has uricase activity. Since the 1922 method gives slightly lower results with blood and plasma filtrates compared with the Folin 1933 method, and essentially the same results when the 1922 method is used for urines, it can be assumed that

[3] The product sold by the Winthrop Chemical Company, Inc., New York, N. Y.

both these methods are measuring mainly uric acid.

Effect of urine flow. With urine flows from 0.83 to 13.9 cc. per minute per 1.73 sq. m., the mean uric acid clearance obtained from 29 clearance periods on 9 subjects averages 15.4 ± 3.2[4] cc. per minute per 1.73 sq. m., with a range of values from 9.0 to 20.6 cc. per minute. The best straight line which describes these data is described by the equation $y = 14.97 \pm 0.067\,x$ with the $S.E._{xy} = 3.21$ cc. The co-efficient of correlation equals 0.076. There is, therefore, no significant correlation between the uric acid clearance and the urine flow. There is also no indication that an augmentation level exists at these urine flows.

This evidence can, therefore, be taken to indicate that the uric acid method used will estimate the uric acid when it is present in very low concentration, since these data confirm previous observations that the uric acid excretion is independent of the urine flow.

Effect of glucose. In order to determine the effect of the injection of a large quantity of glucose upon the uric acid clearance, the patient was given 50 cc. of 50 per cent glucose, intravenously. Either 2 or 3 clearance periods were obtained after a single injection of glucose. The average value for the uric acid clearance (11 clearance periods on 4 different patients) following the intravenous administration of glucose was 15.9 cc. per minute per 1.73 sq. m., with a range of from 8.4 to 20.3 cc. per minute. There is no definite trend to be observed in the uric acid clearance following the injection of glucose. A trend might be expected if the glucose were affecting the uric acid clearance, since it can be assumed that the blood glucose at the time of injection attained a maximum level which constantly decreased. These data indicate that glucose in massive doses has no effect upon the uric acid clearance, since the clearances obtained after the injection of glucose were not significantly different from those obtained with water diuresis.

Effect of diodrast. In another series of 11 patients, an average normal uric acid clearance of 14.0 ± 2.9 cc. per minute per 1.73 sq. m. was obtained. When diodrast was given, as outlined above, to these same patients, on the next day the uric acid clearance averaged 50.6 ± 9.1 cc. The difference between these means, divided by the standard deviation of the means, equals 17.2.

Urea clearances performed on the same specimens of blood and urine averaged for the control day 76.2 ± 13.0 cc. per minute per 1.73 sq. m. and for the day on which diodrast was given, 82.9 ± 12.0 cc. per minute. The difference between these means, divided by the standard deviation of the means, equals 1.66. There is, therefore, no significant difference between the means. These data are presented in Tables II and III.

The creatinine excreted per 30 minutes per 1.73 sq. m. on the control day averaged 24 ± 1.7 mgm. and on the day diodrast was given, 23 ± 2.4 mgm. The difference between these means, divided by the standard deviation of the means, equals 0.66. There is again no significant difference between the excretion of creatinine on the day on which diodrast was given and that on the day diodrast was not given.

The evidence presented shows that diodrast exerts an effect upon the excretion of uric acid. It will be noted that there is no appreciable change in the blood uric acid from one day to the next. The diodrast has no effect upon the excretion of urea and creatinine.

DISCUSSION

The normal uric acid clearance herein reported for the normal postpartum woman is approximately of the same order of magnitude as most of the uric acid clearances reported, or calculated from data reported in that literature on uric acid excretion which presents sufficient data for the calculation of clearances.

The uric acid clearances as calculated by us[5] from the data of Berglund and Frisk (14) on uric

[4] $\sqrt{\dfrac{\Sigma(\Delta)^2}{N-1}}$

[5] Berglund and Frisk do not calculate a clearance. The data for these calculations have been selected from their data. Only those data have been used which were obtained during the first period, before any experimental manipulations were performed. A clearance calculated from the "elimination index" of Berglund and Frisk equals 19.1 cc. per minute (see Bröchner-Mortensen (18 to 20)).

TABLE II

Uric acid clearances

No.	Patient	Treatment	Surface area	Post-partum	V	U	P	U/P	C	Corrected C
			sq. m.	*days*	*cc.*	*mgm. per cent*			*cc. per minute*	
1	D. D.	Control	1.58	5	10.0	6.9	4.9	1.41	14.1	15.4
					11.7	6.8	4.9	1.39	16.3	17.8
		Diodrast		6	10.0	19.8	5.0	3.96	39.6	43.3
					8.7	26.4	5.0	5.3	45.9	50.0
2	M. P.	Control	1.77	5	11.7	4.0	3.1	1.29	15.1	14.7
					15.7	3.7	3.1	1.19	18.7	18.3
		Diodrast		6	17.5	8.7	2.8	3.11	54.4	53.1
					13.2	11.9	2.8	4.25	56.1	54.7
3	R. D.	Control	1.89	6	12.5	3.6	4.7	0.76	9.6	8.8
					7.0	7.4	4.7	1.58	11.0	10.1
		Diodrast		7	15.1	15.1	4.4	3.44	51.7	47.4
					11.6	18.8	4.4	4.27	49.5	45.3
4	C. O.	Control	1.51	5	1.01	48.7	3.3	14.7	14.9	17.1
					0.75	79.7	3.3	21.4	16.1	18.4
		Diodrast		6	8.06	22.3	3.2	6.97	55.6	63.8
					7.43	23.4	3.2	7.30	54.1	62.0
5	C. A.	Control	1.49	5	6.60	5.3	3.3	1.60	10.6	12.3
					5.35	6.3	3.3	1.91	10.2	11.8
		Diodrast		6	10.1	17.4	3.1	5.62	56.6	65.6
					8.0	22.3	3.1	7.20	56.6	65.6
6	A. W.	Control	1.45	6	7.50	7.6	4.3	1.77	13.2	15.7
					3.56	16.0	4.3	3.72	13.3	15.8
		Diodrast		7	6.73	23.2	4.4	5.28	35.4	42.1
					5.46	33.0	4.4	7.50	41.0	48.8
7	G. Mc.	Control	1.91	6	7.28	9.0	6.3	1.43	10.4	9.4
					7.57	9.9	6.3	1.57	11.9	10.8
		Diodrast		7	8.04	32.2	6.4	5.03	40.4	36.6
					6.76	32.7	6.4	5.10	34.6	31.5
8	A. V.	Control	1.69	6	9.86	6.0	3.8	1.58	15.6	15.9
					10.0	5.2	3.8	1.37	13.7	14.0
		Diodrast		7	11.9	14.6	3.8	3.84	45.7	46.6
					6.75	25.7	3.8	6.76	45.6	46.5
9	B. M.	Control	1.45	4	7.23	4.2	3.6	1.17	8.4	10.0
					7.50	5.0	3.6	1.39	10.4	12.4
		Diodrast		5	8.70	18.3	3.3	5.54	48.2	57.6
					9.00	18.6	3.3	5.63	50.6	60.1
10	M. P.	Control	1.70	7	11.5	5.3	4.1	1.29	14.9	15.2
					2.73	18.6	4.1	4.53	12.4	12.7
		Diodrast		8	11.2	17.1	4.1	4.17	46.7	47.6
					2.36	79.0	4.1	19.3	45.4	46.3
11	J. A.	Control	1.71	6	7.43	8.1	4.1	1.98	14.8	15.0
					7.68	8.2	4.1	2.00	15.4	15.6
		Diodrast		7	12.1	15.6	4.1	3.81	46.0	46.4
					11.7	18.2	4.1	4.44	51.9	52.4

TABLE III

Uric acid and urea clearances and creatinine excretion

No.*	Clearance				Creatinine excretion per 30 minutes per 1.73 sq. m.	
	Uric acid		Urea			
	Control	Diodrast	Control	Diodrast	Control	Diodrast
	cc. per 1.73 sq. m.				*mgm.*	
1	15.4	43.3	83.4	86.0	24	26
	17.8	50.0	94.3	75.5	25	24
2	14.7	53.1	70.8	97.7	25	27
	18.3	54.7	93.8	73.6	24	21
3	8.8	47.4	73.5	89.9	24	29
	10.1	45.3	71.8	75.1	24	22
4	17.1	63.6	49.6†	79.4	26	26
	18.4	62.0	47.3†	67.9	25	24
5	12.3	65.6	63.6	106.0	25	23
	11.8	65.6	56.8	97.8	23	22
6	15.7	42.1	73.3	68.5	24	21
	15.8	48.8	72.0	73.1	24	23
7	9.4	36.6	80.1	80.8	23	23
	10.8	31.5	83.6	75.6	23	21
8	15.9	46.6	94.3	87.4	25	21
	14.0	46.5	88.6	73.3	22	20
9	10.0	57.6	53.7	99.9	24	25
	12.4	60.1	66.4	89.5	27	21
10	15.2	47.6	73.6	77.0	24	22
	12.7	46.3	55.0	61.5	21	19
11	15.0	46.4	92.4	97.0	29	22
	15.5	52.4	83.6	90.6	25	27
Average	14.0 ± 2.9	50.6 ± 9.1	76.2 ± 13.0	82.9 ± 12.0	24 ± 1.7	23 ± 2.4

* Correspond with the numbers in Table II.
† Standard clearances. These values are not included in the statistical evaluation of the data.

acid excretion (the uric acid being determined by the Folin 1933 method) averages 23.6 cc. per minute. This figure is a little high. This may be due to the fact that their average plasma uric acid value is 3.16 mgm. per cent which is lower than most values reported for plasma uric acid. An average normal uric acid clearance has also been calculated [6] from Gärdstam's data (15). In this study, the urine uric acid was determined by the Folin and Wu method (16) and the plasma by the Folin 1922 method (10). From 48 clearance periods, on 34 patients with normal kidney function, one obtains an average clearance of 12.9 cc. per minute. Gärdstam reported an average plasma uric acid level of 4.0 mgm. per cent. Coombs *et al.* (2) have also reported a uric acid clearance which averages 11.1 cc. per minute per 1.73 sq. m. in 11 cases. They used the Benedict and Behre (17) method for the determination of uric acid. Their average plasma uric acid value is not reported.

The lowest uric acid clearances (6.93 cc. per minute) have been reported by Brøchner-Mortensen (18 to 20). He has used the reduction of ferricyanide at pH 11 as a method for the determination of uric acid. It should be noted, however, that Brøchner-Mortensen's reported values for serum uric acid are high. They average 6.35 mgm. per cent in 25 normal women and 7.62 mgm. per cent in 25 normal men. These high plasma values (and thereby the low uric acid clearances) may be due to a possible source of error due to glucose, as pointed out by Bulger and Johns (21), in the ferricyanide method used by Brøchner-Mortensen. Bulger and Johns (using a combined ferricyanide and uricase method) report an average plasma uric acid value in the groups which they studied as 3.5 mgm. per cent for females and 4.4 mgm. per cent for males. If one assumes that Brøchner-Mortensen's urine uric acid values are of the

[6] Only those clearances which were performed in the first period in the morning and only those with a urine flow greater than 0.5 cc. per minute were averaged.

correct order of magnitude, it is possible for the purposes of discussion to correct his plasma uric acid values to approximately those reported by Bulger and Johns. Such a correction would increase Brøchner-Mortensen's average uric acid clearance from 6.9 to 12.2 cc. This figure is of the same order as most of those previously reported.

Except then, for Brøchner-Mortensen's value for which there may be an explanation, and the value reported by Berglund and Frisk, the data show a rather good agreement. This agreement actually becomes somewhat enhanced if one considers the low esteem in which uric acid methods have been held.[7] At least 3 different groups of investigators, using different methods for the determination of uric acid have now reported uric acid clearances of the same order of magnitude. It appears probable, therefore, that the normal uric acid clearance lies somewhere between 11 and 15 cc. per minute.

The very high uric acid clearances previously reported from this laboratory are in all probability due to the simultaneous presence and excretion of diodrast. The data presented in the previous paper (1) are, however, still pertinent and any datum in that paper can be compared with any other datum reported in that paper, since the same amount of diodrast was given in each case. The data are, however, erroneous as absolute values. As an approximation, the uric acid clearances can be corrected by dividing by 2.

The data presented herein indicate that diodrast must be added to the list of substances which increase the excretion of uric acid. The mechanism of this reaction or action, however, is still unknown. It is known, however, that diodrast is excreted by the kidney tubule (22). Most probably this excretion inhibits the reabsorption of uric acid. But it is also theoretically possible that uric acid is also excreted by the tubule and the increased activity of the tubule required to excrete the diodrast is reflected in an increased excretion of uric acid. However, we are more inclined to the view that the excretion of diodrast inhibits the reabsorption of uric acid.

It also seems probable that diodrast is affecting one specific mechanism or portion of the kidney tubule or both since the reabsorption of uric acid is not affected by the simultaneous maximal reabsorption of glucose nor is there any significant alteration in the urea clearance or in the creatinine excretion when diodrast is given. These data also indicate that diodrast is excreted by one specific mechanism or portion of the tubule and that any conclusions drawn as to tubular function from the excretion of diodrast may only apply to one portion or mechanism of the tubule. On the other hand, it may be possible that larger amounts of diodrast than those given in these experiments may also have some effect upon the urea clearance.

[7] Apparently, many investigators consider them both inadequate and inaccurate. This certainly cannot be attributed to any lack of effort in this direction. One of us (R. W. B.) has collected a bibliography of 280 titles of papers dealing with the quantitative determination of uric acid in blood and urine.

SUMMARY

The uric acid clearance as determined in the normal woman, 1 to 8 days postpartum, averaged 15.4 ± 3.2 cc. per minute per 1.73 sq. m. in a series of 9 subjects, and 14.0 ± 2.9 cc. per minute per 1.73 sq. m. in another series of 11 subjects. There is no significant difference between these values. The injection of diodrast resulted in an increased excretion of uric acid. The average uric acid clearance in the 11 subjects described above increased to 50.6 ± 9.1 cc. per minute per 1.73 sq. m. when a given amount of diodrast was being excreted simultaneously. The diodrast did not have any effect upon the urea clearance or the creatinine excretion. In contrast, the injection of hypertonic solutions of glucose exerted no effect upon the excretion of uric acid.

Dr. H. W. Smith has recently called our attention to a chapter on gout by J. H. Talbot in Oxford Medicine, vol. IV, part I, chapter IV, Oxford University Press, New York, 1943, in which a similar effect of diodrast upon uric acid is described.

We wish to express our appreciation and thanks for the aid and suggestions received during the course of this work from Dr. H. J. Stander and Dr. Vincent du Vigneaud. The nursing staff of the Lying-In Hospital has been most co-operative in assisting in the collection of blood and urine specimens. Particular thanks are extended to Miss L. Woermbke, R.N., Miss A. Klubko, R.N., Mrs. M. Carter,

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A METHOD OF DISTRIBUTING BETA-RADIATION TO THE RETICULO-ENDOTHELIAL SYSTEM AND ADJACENT TISSUES[1]

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In this study, an attempt has been made to measure the accumulation and retention of radioactive particulate matter by macrophages, and to determine the effects of various levels of β-radiation from phosphorus upon these cells and the tissues intimately associated with them. Quantitative studies of phagocytosis (1 to 4), and phagocytosis of radioactive material have received some attention (5 to 11), but as far as we are aware, this study is the first in which a substance emitting only β-radiation has been distributed to the reticulo-endothelial system and its effects noted. Recently, radio-bromine has been used in synthesizing radioactive di-brom trypan blue and the distribution of this compound studied after injection into rabbits and mice (5, 7).

PREPARATION OF RADIOACTIVE CHROMIC PHOSPHATE

Anhydrous chromic phosphate has been used in this study because of its inertness and apparent lack of toxic effect; it is easily prepared in a fine and even suspension of particles, 1 μ or less in diameter. Radioactive phosphorus (P^{32}) was obtained as a solution of di-sodium phosphate with non-radioactive phosphorus carrier. An equivalent amount of sodium bicarbonate and chromic nitrate solution in excess was added. The freshly precipitated salt was a crystalline hydrate, readily soluble in hot water, acids, or bases. This was centrifuged, washed, and recentrifuged. After complete drying at 110° C., the product was heated to 600° C. in the electric furnace. This final treatment resulted in a dark brown, amorphous powder, insoluble in strong acid or alkali, or in aqua regia. The product was transferred to a 50 ml. serum bottle, half filled with pyrex beads. A suitable quantity of isotonic glucose solution was added (1 to 2 ml. per 100 mgm.) and the bottle autoclaved. Rotation of the bottle in a mill for 12 hours, at a speed which allowed the beads to cascade down the sides, resulted in a preparation suitable for intravenous administration. The final product has the appearance of thin clay mud.

Another method of preparing the chromic phosphate has been used recently; it gave better precipitation of the phosphorus: Every volume of 0.1 N Na_2HPO_4 was mixed with one volume of 0.5 N $Cr(NO_3)_3$ and one-third volume of 1.0 N NaAc. The crystalline hydrate obtained was treated as above.

EXPERIMENT I

Two groups of mice were used to obtain information regarding the uptake and retention of chromic phosphate and the effects of its β-radiation.

In *Group A*, 12 female Swiss mice received varying doses of radioactive chromic phosphate via the tail vein, and were allowed varying survival periods (Table I).

In *Group B*, 16 female Swiss mice, divided into 4 groups, were injected through the tail vein with 3.4 mgm. of chromic phosphate, suspended in 0.25 ml. of isotonic glucose. All of the animals received the same weight of salt, but the samples were so prepared that the radioactivity in each sample varied. Thus, Group 1 received 70.8 microcuries per mouse; Group 2, 39.4 microcuries; Group 3, 18.2 microcuries; and Group 4, 4.25 microcuries. Two animals from each group were sacrificed after 3 days and the remainder after 5 days, with 2 control animals for each period.

Samples of spleen, liver, and lung were taken from mice 6 to 12 (Table I) for radioactive and histologic examination while in mice 1 to 5, only the liver was examined radioactively. Tissue samples of 20 to 150 mgm. wet weight were taken for radiologic analysis on a β-sensitive Geiger counter tube and the Lauritsen electroscope (14). Results listed in Table I were calculated as percentage of the dose recovered in each tissue; for comparing the activity in different organs, percentage of recovery per gram is also given.

There are several difficulties attending the conversion of internal β-radiation to comparable roentgen (r) values. A large block of tissue, in which one microcurie of radioactive phosphorus is distributed uniformly in each gram of tissue, absorbs 3,550 ergs per gram of tissue per day. This is equivalent to 42 r of x-rays (12). From a given source in an aqueous media, about 48 per cent of β-radiations of P^{32} are absorbed in each 0.1 cm. distance traversed.[2] Since the mouse liver and spleen are only 0.2

[1] Partial support of this work was provided by the Columbia Fund for Medical-Physics. (Columbia Foundation).

[2] From P^{32} β-ray absorption curves with aluminum (13).

TABLE I

Group A—12 mice receiving varying doses of radioactive chromic phosphate

Animal number	Body weight	Duration of experiment	Final body weight	Chromic phosphate			Liver			Spleen			Lung		
				Volume of dose			Uptake per gram of tissue	Uptake whole organ	Total r‡ accumulated	Uptake per gram of tissue	Uptake whole organ	Total r‡ accumulated	Uptake per gram of tissue	Uptake whole organ	Total r‡ accumulated
	grams		*grams*	*ml.*	*mgm.*	*micro-curies**	*per cent*	*per cent*		*per cent*	*per cent*		*per cent*	*per cent*	
1		24 hours		0.10	0.26	3.75	99								
2	18.0	24 hours	18.0	0.10	0.26	3.75	72								
3	21.5	24 hours	21.5	0.10	0.26	3.75	140								
4	24.0	24 hours	24.0	0.10	0.26	3.75	63								
5	23.0	108 hours	23.0	0.50	1.3	18.8	83								
6	20.5	30 days	20.0	0.50	1.3	18.8	90	81	4,270	49	4.3	1,140	15.7	2.6	88
7	20.5	10 days	16.0	0.50	20.0	230	78	59	39,800	50**	4.9	9,650	6.3	0.63	1,470
8	20.0	15 days	19.0	0.25	10	115	57	65	20,800	54**	6.2	9,000	9.0	1.4	1,500
9	21.5	15 days	21.0	0.25	10	115	61	77	22,200	58**	7.0	9,300	8.0	1.9	1,330
10	20.5	5 days	20.0	0.25	10	265	70	69	24,500	98**	11.7	15,800	7.2	1.3	2,070
11	24.0	23 days†	18.0	0.25	10	265	70	67	52,300	60**	13.1	20,200	12.1	2.7	3,950
12	22.5	23 days†	17.0	0.25	10	265	77	72	57,500	50**	14.7	17,000	33.5	6.6	11,300

* Microcuries with uranium X_2 standard = 3.7×10^4 primary β-particles per second.
** Based on an estimated normal spleen weight of 120 mgm.; observed weight, 7 to 13 mgm.
† Died.
‡ In β-radiation (see text).

to 0.6 cm. thick, an appreciable and varying portion of the β-rays will be absorbed outside the liver and spleen. A graphic model of the liver elliptically shaped (2.9, 2.6 cm. diameters) and 0.60 cm. thick in the middle tapered to the edge, gave an average distance of 0.2 cm. traversed by β-particles of internally produced β-radiation. The thin periphery of the liver and the spleen will absorb less (20 to 50 per cent). Near the center of the liver, the energy absorbed will be close to 90 per cent or 38 r per microcurie per gram of tissue per day (Figure 1). The liver on the average will receive $42 \times 0.73 = 30.7$ r per microcurie per gram of tissue per day. Estimation of the spleen is more difficult because of its variation in size and also in position with respect to the liver. A conservative estimate for the spleen and lungs is 30 to 50 per cent self-absorption. The r values in Table I and in the text are based upon the constants: 30.7 r per microcurie of P^{32} per gram of liver per day and 17 r for the lungs and spleen. The values in Table I are corrected for the decay of P^{32} and are accumulated to the termination of each observation.

Results

None of the mice used in these 2 groups showed untoward symptoms during or after injection. Quantitative measurements of the uptake of the suspension agreed substantially with those obtained by other workers on similar problems (1, 2, 9). The bulk of the material was found in the liver, with similar quantities per gram of tissue in the spleen. The lungs showed a smaller uptake than these organs. Considerably more chromic phosphate is found in the liver than in the similar case of azo-dye administration (7). In the case of one mouse, allowed to survive 30 days after injection, 81 per cent of the dose administered was retained in the liver.

Livers examined macroscopically always showed varying degrees of color change, depending upon the amount of material administered. The opaque, mud-colored livers are evidence of their chromic phosphate content. Histologic examination of the liver revealed that almost all of its chromic phosphate content could be accounted for in the endothelial cells of the sinusoids. Rarely, a few granules were seen within the hepatic cells. Except for an occasional macrophage showing nuclear pyknosis, none of the cells, even when heavily laden with the chromic phosphate, appeared to be injured. The spleen, on the other hand, showed a reaction in keeping with the known high radiosensitivity of lymphoid tissue. In the animals of *Group B*, splenic weights were found at necropsy to vary inversely as the total dose of radiation administered. In animals 6 to 12 of *Group A* (Table I), which had received a considerably higher dosage, the spleens were reduced almost to a connective tissue framework and macrophages.[3] As in the liver, the radioactivity found

[3] A 4.5 kilogram rabbit was given 4 ml. of chromic phosphate suspension (total dose 800 microcuries and 60

in the spleen could be accounted for by the chromic phosphate granules in individual and fused macrophages. Except for the presence of sparsely distributed macrophages bearing chromic phosphate particles, the lungs appeared normal, there being no evidence of parenchymal damage due to the β-radiation.

All animals which received doses greater than 20 microcuries showed an 80 to 95 per cent decrease in spleen size by the fifth day. The general loss of weight of *Group A* animals 7 to 12 is noted in Table I. Animals in this series receiving more than 100 microcuries incurred a 25 per cent weight loss.

EXPERIMENT II

Five female mice, *Group C*, weight 20 to 23 grams, were injected with a tracer dose of chromic phosphate of 1 mgm. in weight. These mice were sacrificed at 5 days and the various tissues were assayed for radioactivity as in Experiment I (Table II).

A female dog, weight 8.5 kgm., was given a comparable dose of 30 microcuries and 85 mgm. of $CrPO_4$. The dog was sacrificed at the end of 4 days. Liver, spleen, lungs, red bone marrow, lymph nodes, and pituitary were removed whole, weighed, and ashed at 500° C. Results are tabulated in Table III.

Results

In the dog and mice, as much as 90 per cent of the total injected chromic phosphate suspension can be recovered from the liver in 4 to 5 days. In both animals, the liver, lungs, and spleen account for the major part (95 to 98 per cent) of the total injected material. An almost negligible amount is distributed in the other tissues.

In comparison to *Group A*, Table I, there may

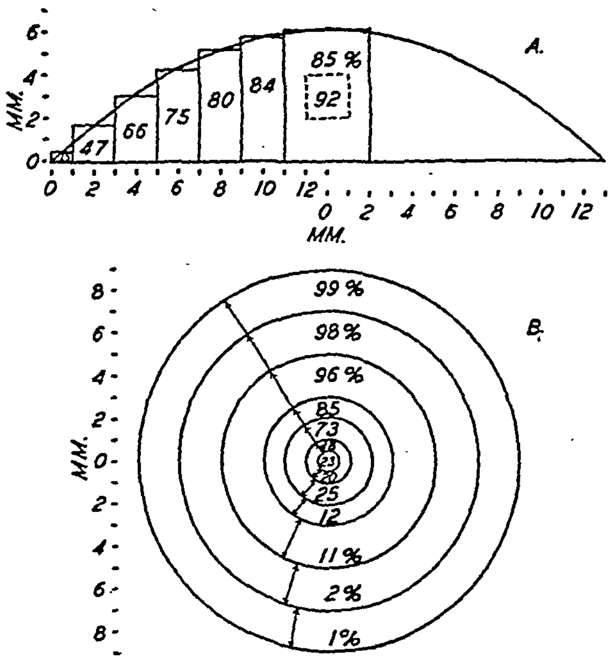

FIG. 1

A. CROSS-SECTION OF WHOLE MOUSE LIVER (WEIGHT 1.1 GRAMS) RESTING ON A FLAT SURFACE

The segments are labeled according to the average percentage of radiation absorption by the whole liver of β-rays originating in that segment.

B. THE CONCENTRIC RINGS ARE THE EXPECTED ABSORPTION OF THE β-RAYS AT VARIOUS RADII FROM A POINT SOURCE

Consider the center of "B" to occur anywhere in "A" and that both have solid dimensions. In the 85 per cent volume in "A," the average of all possible point sources of radiation will travel so that 85 per cent will be absorbed in the liver. It can be determined directly from the diagram that if the "B" is superimposed upon "A" so that the centers of each coincide, then 92 per cent of the effective absorption area of "B" will be covered by "A" (see dotted area in center of "A").

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mgm. of chromic phosphate). After 30 days the spleen was examined at biopsy; at that time it was 5 cm. wide and 10 cm. long. By 60 days, no changes in body weight had occurred; there were no macroscopic changes in any abdominal or thoracic viscera, except the spleen. Of the spleen, there remained only a small pencil-shaped sac filled with a little fluid and chromic phosphate.

TABLE II

Group C—Percentage of recovery of $CrPO_4$ per gram of tissue 5 days after 1 mgm. dose of $CrPO_4$

Whole liver	Liver	Lung	Spleen	Femur	Lymph nodes	Thymus	Kidney	Salivary gland	Skin and subcutaneous C.T.	Blood	Muscle	Intestine
81	83	37	32	3.3	1.1	1.1	1.0	0.80	0.7	0.11	0.1	
85	90	15	49	2.3	0.6		0.7				0.02	
90	99	26	50	3.0	0.4		0.8		0.6	0.1		1.0
91	89	29	45	2.7	0.5		0.5		0.9	0.1		
89	92	30	47	1.5	1.0		0.9					

TABLE III

Recovery of $CrPO_4$ in an 8.5 kgm. dog, female; dose 85 mgm. and 30 microcuries of $CrPO_4$

Organ	Recovery per 100 grams of tissue	Recovery per whole organ
	per cent	*per cent*
Liver	29.1	90.2
Spleen	18.4	6.3
Lungs	1.0	1.6
		98.1
Red bone marrow	0.35	
Lymph nodes	0.45	.... 1.9
Pituitary	0.26	
All others		

be some reason for expecting the phagocytic efficiency of the liver to be decreased with the larger dosages of chromic phosphate. The percentage of recovery of a dose of 10 mgm. or greater per mouse is lower than that observed with doses of 1 mgm. per mouse. This might have been a result of partial blockage of the liver endothelial cells. It is also possible that the greater concentration of the chromic phosphate suspension given to *Group A* animals 7 to 12 may have resulted in a different particular aggregation pattern of the chromic phosphate when injected into the blood stream.

EXPERIMENT III

Two more groups of mice were employed in order to obtain information regarding (*1*) the effects of high levels of β-radiation, over longer periods of time, and (*2*) the effects of prolonged retention of large amounts of inert chromic phosphate in the body. In *Group D*, comprising 24 female "A" strain mice, weight 25 to 27 grams, 10 animals were injected intravenously with 9.0 mgm. of radioactive chromic phosphate (300 microcuries), 6 with 3.6 mgm. (120 microcuries), 5 with 1.8 mgm. (60 microcuries), and 3 served as controls. All animals were sacrificed at the end of 3 months. In *Group E* "A" strain male mice, weight 19 to 23 grams, 12 mice received intravenously 20 mgm. of inert (non-radioactive) chromic phosphate and 11 others received 13 mgm. Fifteen untreated animals served as controls. Three mice on each level and 3 controls were sacrificed at the end of 3, 7, and 12 months.

Results

All but 2 of the animals of *Group D* appeared in good condition at the end of 3 months. Growth had continued and the mice appeared normal in every respect. Radioactive analyses were not made, but it was estimated on the basis of data obtained from Experiment I that the mice in *Group D* on the highest dosage received a minimum of 81,000 r in the liver and spleen and 12,000 r in the lungs. These organs in the mice on the 2 lower dosages of radioactive material received proportionately less irradiation. Since the half-life of the P^{32} used in preparing the chromic phosphate was approximately 14.5 days, only about 1 per cent of the activity could be expected to remain after 3 months.

Histologic examination of the liver revealed that in the 10 animals which had received 300 microcuries in 9.0 mgm., much of the chromic phosphate had been retained in the Kupffer cells. In the animals of the 1.8 mgm. dose, little or no chromic phosphate could be detected. In all but 2 animals of the 2 groups, the livers appeared normal except for the phagocytized material. These 2 mice which had received the high radioactive dosage showed a yellow tingeing of the skin suggestive of liver damage, and at the end of the experiment, their weights were significantly less than the group average. Necropsy revealed large livers which, on histologic section, were diagnosed as hepatomata. No metastases were found. In several thousand autopsies of mice of this strain, this type of tumor had not previously been encountered.

The spleen of all the animals in *Group D* (3 months after injection) appeared normal in size and weight, and on microscopic section, it was found that complete restoration to normal organization had occurred after the destructive effects of the radiation had disappeared. The lungs of all animals but one were of normal appearance. The right lung of this animal was capped at the apex by a firm white nodule which, on microscopic examination, was diagnosed as a bronchiogenic carcinoma. No other evidence of neoplasm was found in this animal, and since on 2 subsequent occasions, similar nodules have been found in normal mice, they probably have no relation to the radioactive material injected.

All of the animals of *Group E* which had received large quantities (13 had 20 mgm.) of inert chromic phosphate seemed normal in appearance, growth, weight, and activity. All of the mice when autopsied after 3, 7, and 12 months, respectively, showed chromic phosphate microscopically in the lungs, spleen, and particularly in the Kupffer cells of the liver. Here,

many of the cells had aggregated into clumps of from 5 to 20 cells in the sinusoids or in the connective tissue surrounding the larger vessels. Lymphoid infiltration was sometimes noted in these perivascular sites and was, in some instances, accompanied by moderate numbers of neutrophilic leukocytes. In one of the livers, areas of definite necrosis were observed, but these were small and replaced in part by connective tissue. Except for the widely scattered deposits of chromic phosphate which could be observed grossly as well as microscopically, the livers seemed to be essentially normal.

DISCUSSION

The information obtained on the uptake and retention of radioactive phosphorus in the form of an insoluble chromic salt differs strikingly from that gathered in studies on mice in which soluble radioactive phosphorus was given (14). In the latter work, the liver, kidney, and small intestine contained about 9 per cent per gram of tissue and other soft tissues ranged downward to 1 per cent per gram or less. In such cases, the initial activity was quickly dissipated by excretion and interchange with the phosphate stores in the animal. The high degree of retention of radioactive chromic phosphate and the tolerance of the animal to dosages as large as 1 mgm. per gram of body weight indicate that, except for the β-radiation, the radioactive chromic phosphate is inert. That this retention is partly, if not wholly, attributable to the insolubility of the form of chromic phosphate used seems to follow from the results obtained by Drinker *et al.* (1, 2). These workers believed that the varying degree of retention by the body of different suspensions studied by them was a reflection of their relative solubilities.

The finding of large amounts of the inert chromic phosphate in the spleens and livers of the mice 12 months after injection would suggest that this insoluble material is released slowly from the animal.[4] That it may be released from certain dying or dead macrophages and be phagocytized again by other healthy ones seems likely. However, there is no evidence that it could be "leached out" of the reticulo-endothelial cells and used elsewhere or excreted as readily as when colloidal calcium phosphate is injected (3, 4).

No evidence was obtained of any serious damage caused to mice by the retention of the relatively enormous doses of non-radioactive chromic phosphate over a long period of time. In this respect, it can be best compared to carbon ingested by the pulmonary macrophages. The radioactive chromic phosphate, even in low doses, caused rapid involution of the lymphoid elements associated with the reticulo-endothelial cells bearing the radioactive granules, but, as far as could be determined, the liver and lung parenchymal cells escaped damage except in the 2 mice (Experiment III, *Group D*) receiving the highest dosage of radioactivity (300 microcuries). It is not unlikely that the radioactive chromic phosphate was responsible for these 2 tumors and further experiments are now in progress to decide this question.

There may be considerable variation in tolerance to β-radiation in mice. The 10 mice receiving 300 microcuries in Experiment III lived 3 months in relatively good condition, while mice 11 and 12, receiving 265, died in 23 days.

SUMMARY

Suspensions of anhydrous chromic phosphate were prepared from both radioactive and non-radioactive phosphorus. The average particle size was 1 micron, and the suspension in isotonic glucose was suitable for intravenous injection.

Chromic phosphate is inert and insoluble in the living tissues. Doses as large as 1 mgm. per gram of body weight are tolerated in mice.

Quantitative radioactive determinations of the uptake of intravenously injected radioactive chromic phosphate were made in the mouse and the dog. Approximately 90 per cent of the material was found in the liver. The bulk of the remaining portion was found in the spleen and in the lungs. This agreed with histologic findings.

The chromic phosphate is retained in the endothelial cells of mice for at least 1 year without detected decrement.

[4] Presence of minute traces of radioactivity in the bile, feces, and urine was detected in animals injected with chromic phosphate of high activity, thus indicating a very slow but continuous excretion via these pathways.

The pattern of phagocytosis of injected chromic phosphate particles brings about a concentration of β-radiation in the liver and spleen which is of the order of 100 times the concentration in other tissues, except in the lung tissue. In the mouse, however, about 27 per cent of the β-radiation originating in the liver and spleen is absorbed by adjacent tissues up to 0.60 cm. distance. In larger animals, where the shortest thickness of these organs is 1 cm., relatively little external loss will result.

The lymphoid tissue of the spleen was decidedly sensitive to the radioactive chromic phosphate. Mice that did not die from large doses of β-radiation showed rapid regeneration of the spleen.

Reticulo-endothelial cells, hepatic and pulmonary epithelium are able to withstand large doses of β-radiation. However, in 2 mice, primary hepatoma developed and this may have been due to the high dosage of β-radiation (a magnitude of an accumulation of 80,000 r in 20 days) in the liver.

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THE ABSORPTION AND EXCRETION OF PENICILLIN FOLLOWING CONTINUOUS INTRAVENOUS AND SUBCUTANEOUS ADMINISTRATION

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Penicillin, a product of the growth of a fungus, *penicillium notatum*, has become established as a therapeutic agent of value in the management of various infections, particularly those caused by the pyogenic cocci (1).

Because penicillin is unstable in the presence of acid, it may not be given by mouth and absorption from the duodenum is imperfect. It has, therefore, been administered parenterally in nearly all of the recorded clinical studies. Three routes have usually been used: (*1*) intermittent intravenous; (*2*) intermittent intramuscular; and (*3*) continuous intravenous. Adequate clinical results have been obtained with each but in only one instance have studies been made of the fate of the injected material.

It has been shown (2) that the injection of a single large dose of penicillin, intravenously, was followed by a very rapid rise and fall of the blood level of this substance, so that at the end of 2 hours, the amount remaining was not detectible by the method used. During this interval, about 60 per cent of the injected penicillin appeared in the urine. Absorption was somewhat slower and the blood levels were lower but better maintained following intramuscular administration. Absorption from the subcutaneous tissues was irregular, and the levels reached in the blood were low.

The use of the continuous intravenous drip for the administration of penicillin has been advocated (3). This author has suggested a daily dose of 50,000 units, but has not studied the concentrations of the drug in the blood or urine.

It is the purpose of this report to describe the results of determinations of blood and urine concentrations of penicillin obtained during the study of patients under treatment with this agent, all of whom received penicillin by continuous intravenous or subcutaneous drip.

METHODS AND MATERIALS

Penicillin concentration. The concentration of penicillin in plasma and urine was determined routinely by a modification of the method previously described (4). In certain instances, the plasma concentration was measured by a photoelectric turbidimetric technic. Both of these methods will be described in detail elsewhere. It is important to bear in mind that both involve dilution technics and are, therefore, subject to considerable error. The exact magnitude of this error has been studied and will be described elsewhere, but may be stated to be approximately ± 30 per cent.

Blood. Since it has been previously shown (2) that nearly all of the blood penicillin is dissolved in the plasma and very little in the red blood cells, the former material was used in all tests, whole blood being collected aseptically into tubes containing an appropriate amount of sterile sodium citrate. Preliminary tests revealed that this substance does not interfere with the action of penicillin.

Urine. Timed urines were collected, frequently by catheter, and immediately sterilized by Seitz filtration. The concentration of penicillin in each specimen was determined by the modification of the Rammelkamp method mentioned above.

Penicillin solutions.[1] The sodium salt of penicillin, obtained as a powder, dehydrated and *in vacuo*, was dissolved in appropriate amounts of sterile, pyrogen-free isotonic sodium chloride, or 5 per cent dextrose solution. The concentrations most frequently used contained 25, 100, and 200 units per cc.

Administration. Penicillin solutions of suitable concentration were administered by continuous intravenous or subcutaneous drip in the usual way. Not more than 1,000 cc. were given in 24 hours by the subcutaneous route.

RESULTS

Blood levels and urinary excretion

A. *Carefully controlled intravenous administration*

A series of experiments was undertaken to determine the plasma levels and the rates of

[1] The penicillin was provided by the Office of Scientific Research and Development from supplies assigned by the Committee on Medical Research for clinical investigations, recommended by the Committee on Chemotherapeutic and Other Agents of the National Research Council.

urinary excretion of penicillin following its administration by the continuous intravenous route at a carefully controlled rate.

Seven patients, all afebrile primary or secondary syphilitics, were well hydrated for 24 hours before the experiment was begun. The administration of penicillin was started in the morning, with the patient under basal conditions until the conclusion of the experiment. Solutions of penicillin in normal salt solution of a concentration of 25 and 100 units per cc. were prepared and administered by the continuous intravenous route in such a way that 2,500, 5,000, 10,000, and 20,000 units per hour were given at a rigidly controlled rate. Each level was maintained for a 2-hour period, during the last 30 minutes of which a blood and a timed urine specimen were obtained. The results of these experiments are presented in Table I.

It will be observed that the results are, on the whole, quite consistent in the light of the inherent error in the methods of determining the concentration of penicillin. The latter factor makes it most profitable to discuss these experiments on the basis of the last section of the table, which presents the average of all the values obtained in 7 experiments.

Plasma concentration. The plasma concentration of penicillin at the various rates of administration was determined in 4 instances by

TABLE I

Absorption and excretion of penicillin following continuous intravenous administration

Name	Penicillin administered	Concentration of penicillin			Urine excretion	Excretion of penicillin		Plasma cleared of penicillin (dilution)	Plasma cleared of penicillin (turbidimetric)
		Plasma dilution	Plasma turbidimetric	Urine dilution					
	units per hour	*units per cc.*	*units per cc.*	*units per cc.*	*cc. per minute*	*units per minute*	*units per hour*	*cc. per minute*	*cc. per minute*
Fr	2,500	0.04	0.04	4.0	7.8	31.2	1,875		
	5,000	0.1	0.14	60.0	1.7	102.0	6,100	1,020	
	10,000	0.2	0.25	200.0	1.4	280.0	16,800	1,400	
	20,000	0.5	0.48	100.0	4.5	450.0	25,000	900	
Br	2,500	0.04	0.04	4.0	6.2	24.8	1,490		
	5,000	0.06	0.06	20.0	4.3	86.0	5,150	1,430	
	10,000	0.10	0.12	20.0	5.0	100.0	6,000	1,000	
Le	2,500			5.0	8.7	43.5	2,650		
	5,000	0.06		10.0	6.5	65.0	3,900	1,080	
	10,000	0.06		50.0	2.0	100.0	6,000	1,670	
	20,000	0.20		20.0	7.2	144.0	8,650	720	
Gr	2,500	0.06	0.07	5.0	7.7	28.5	2,310	640	
	5,000	0.10	0.11	50.0	2.6	130.0	7,800	1,300	
	10,000	0.20	0.25	20.0	11.0	220.0	13,200	1,100	
	20,000	0.50	0.32	100.0	5.2	520.0	31,200	1,040	
De	2,500	0.06	0.06	5.0	11.7	58.5	3,500	970	
	5,000	0.10	0.12	6.0	13.7	82.0	4,920	820	
	10,000	0.20	0.36	5.0	15.2	76.0	4,560	380	
	20,000	0.50	0.42	20.0	15.4	308.0	18,500	615	
En	2,500	0.05		5.0	6.1	30.5	1,830	610	
	5,000	0.10		6.0	8.3	49.8	2,980	498	
	10,000	0.20		20.0	10.6	212.0	12,700	1,060	
	20,000	0.40		40.0	16.9	676.0	40,500	1,690	
Sm	2,500	0.05							
	5,000	0.06		6.0	18.5	111.0	6,660	1,850	
	10,000	0.10		10.0	14.5	145.0	8,700	1,450	
	20,000	0.20		10.0	16.1	161.0	9,660	805	
Average values	2,500	0.05	0.05			37.8	2,270	755	755
	5,000	0.08	0.11			89.5	5,370	1,120	815
	10,000	0.15	0.24			162.0	9,720	1,080	735
	20,000	0.38	0.41			376.0	22,580	989	915

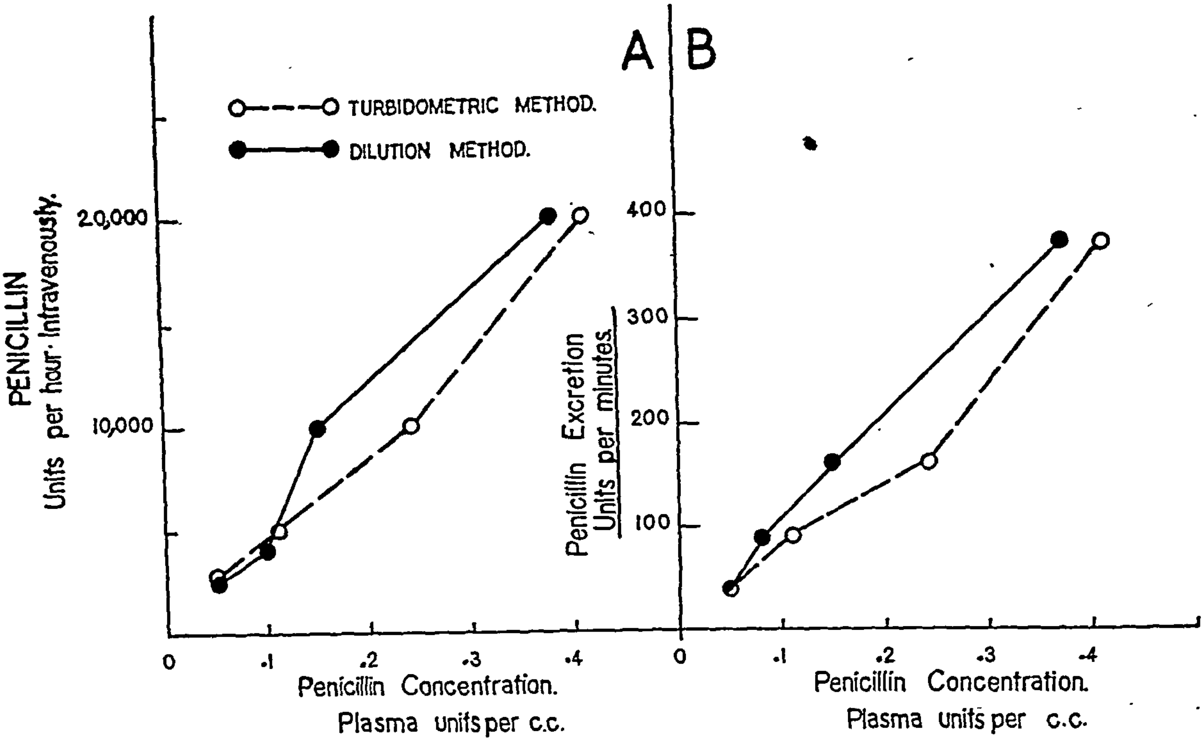

FIG. 1. A. RELATIONSHIP BETWEEN THE AMOUNT OF PENICILLIN INJECTED AND THE PLASMA CONCENTRATION
B. RELATIONSHIP BETWEEN THE RATE OF URINARY EXCRETION OF PENICILLIN AND THE PLASMA CONCENTRATION

the turbidimetric method and in 7 by the dilution technic. It will be observed that there were large variations between the plasma levels obtained in different subjects, but that there is a close correlation between the average values obtained by the two methods.

The continuous intravenous infusion of 2,500 units of penicillin per hour induced a concentration of this substance in the plasma of 0.05 units per cc.; 5,000 units, 0.08 to 0.11 units per cc.; 10,000 units, 0.15 to 0.24 units per cc.; and 20,000 units, 0.38 to 0.41 units per cc.

Figure 1A, in which the plasma concentrations obtained by the two methods are plotted against the rate of administration, reveals that there is an approximately linear relationship between these two factors.

Urinary excretion. The data presented in Columns 6 and 7 of Table I indicate that penicillin is rapidly excreted in the urine. Figure 1B demonstrates that the rate of excretion is proportional to the plasma concentration. The average hourly rates of excretion are equal to the amount administered. No evidence of destruction of penicillin in the tissues was discovered.

Plasma clearance. In Columns 8 and 9, the average amounts of plasma cleared of penicillin by the kidneys, in cc. per minute, are presented. The calculations have been made using the plasma concentrations obtained by the dilution and turbidimetric methods separately.

From 755 to 1,120 cc. of plasma were cleared per minute. These are rates comparable to those obtained when studies have been made of substances believed to be excreted by the renal tubules and are many times greater than the clearance of 130 cc. per minute to be expected by glomerular filtration alone (5).

The plasma clearances of penicillin at the lowest and highest rates of administration were comparable, suggesting that the maximum rate of tubular excretion, or Tm (5), of this substance had not been reached.

It is also of interest to point out that several-fold differences in the rate of urinary flow, in certain subjects, were not associated with varia-

TABLE II

Excretion of penicillin in febrile patients

Patient number	Dose	Route	Temp.	Penicillin		Urine excretion	Penicillin excretion		Penicillin plasma clearance
				Plasma	Urine				
	units per hour		°C.	*units per cc.*		*cc. per minute*	*units per minute*	*units per hour*	*cc. per minute*
1	4,160	Subcut.	38.5	0.033	5.0	4.1	20.5	1,230	620
2	8,320	Subcut.	39.0	0.10	10.0	4.7	47.0	2,820	470
3	8,320	Intraven.	39.0	0.14	33.0	8.0	260.0	15,600	1,850
4	8,320	Intraven.	38.5	0.20	70.0	6.5	455.0	27,300	2,270
5	8,320	Intraven.	38.5	0.20	40.0	8.5	350.0	21,000	1,750
6	8,320	Subcut.	38.5	0.10	10.0	24.2	242.0	14,550	2,420
Average									1,560

tions in the plasma clearance of penicillin. This is in accord with observations made on other materials known to be excreted by the renal tubules.

B. *Effect of fever*

It has been shown that the plasma clearance of substances excreted by the renal tubules is closely related to the rate of blood flow to the kidney and that the latter may be increased by the injection of pyrogens (6). It was, therefore, of interest to determine the rate of excretion of penicillin in febrile patients. The results of 6 such experiments are presented in Table II. In 3 patients, the material was administered by continuous subcutaneous drip and, in 3, by continuous intravenous drip.

Definite conclusions cannot be drawn from these few observations, but it should be pointed out that the average plasma clearance in the presence of fever was approximately 50 per cent greater in these febrile individuals than in the previously described afebrile group.

C. *Plasma levels during routine administration*

The concentration of penicillin in the plasma following its administration at rigidly controlled rates by the continuous intravenous route has been described. Many determinations were also made of the amount of penicillin in plasma, obtained following the administration of this agent, intravenously and subcutaneously in various amounts, when patients were under routine treatment. At such times, the rate of infusion of the penicillin solution was under the careful supervision of experienced nurses and ward officers, but was not controlled as it was in the previous studies.

The results of these observations are presented in Table III. Considerable variation was discovered to exist between determinations of the

TABLE III

Plasma concentrations of penicillin obtained during routine administration by continuous intravenous and subcutaneous drip

Penicillin dosage in 24 hours	Plasma concentration			
	Individual determinations		Average	
	Intra-venous	Sub-cutaenous	Intra-venous	Sub-cutaneous
	units per cc.			
100,000 units	0.40	0.05		
	0.20	0.06		
	0.10	0.05		
	0.10	0.04		
	0.20	0.03		
	0.10	0.05		
	0.06	0.05		
	0.04	0.05		
	0.04	0.06		
		0.06	0.13	0.05
200,000 units	0.10	0.10		
	0.20	0.05		
	0.33	0.10		
	0.14	0.20		
	0.20	0.10		
	0.20			
	0.20			
	0.40			
	0.50			
	0.10			
	0.10		0.22	0.11
400,000 units	0.20			
	0.50			
	0.40			
	0.60		0.42	

plasma level in different patients and in the same individual at different times. It will be observed that the average plasma level induced by the administration of 100,000 units intravenously in 24 hours was 0.13 units per cc.; 200,000 units, 0.22 units per cc.; and 400,000 units, 0.42 units per cc. These values closely approximate those obtained when penicillin was given at rigidly controlled rates by the same route.

The infusion of penicillin subcutaneously was not associated with the development of plasma levels of this magnitude. The average concentration obtained during the administration of 100,000 units in a 24-hour period by this route was only 0.05 units per cc.; during the administration of 200,000 units, only 0.11 units per cc.

DISCUSSION

The observations just described have demonstrated that predictable plasma concentrations of penicillin will be obtained when penicillin is administered by continuous intravenous drip under rigidly controlled conditions. The infusion of 2,500 units per hour (60,000 units per day) may be expected to induce a concentration of approximately 0.05 units per cc.; 5,000 units per hour (120,000 units per day), 0.10 units per cc.; 10,000 units per hour (240,000 units per day), 0.20 units per cc.; 20,000 units per hour (480,000 units per day), 0.40 units per cc. When studies were made of plasma levels obtained during the routine treatment of patients by the intravenous route, they were found to approximate these values closely.

If penicillin was administered subcutaneously, the plasma levels were about 50 per cent lower than those observed during the treatment of patients by the intravenous route. The reason for this discrepancy is not apparent but it is possible that the creation of a reservoir of penicillin in the subcutaneous tissues permits its destruction before it is absorbed.

These studies have also clarified the mode of excretion of penicillin. It has beensuggested (7) that the renal tubules must play an important rôle in the clearance of penicillin from the plasma and blocking of the tubular excretory mechanism by the simultaneous injection of diodrast and penicillin has been attempted, with partial success.

The calculations presented above, based upon studies in afebrile patients under basal conditions, in whom from 750 to 1,120 cc. of plasma were cleared of penicillin per minute, definitely proves the tubular excretion of this substance, since the average amount cleared by glomerular filtration alone could have been only 130 cc. per minute (5).

These values are higher than those obtained for the average diodrast clearance, 688 cc. per minute (5), and suggest that the intravenous injection of penicillin may increase the renal blood flow in a manner similar to that induced by the injection of pyrogens (6). It is not proper, however, to draw this conclusion from so few observations, uncorrected for surface area or sex. It is logical to assume, however, that the renal blood flow and the penicillin plasma clearance will be increased in the febrile patient. That this is the case is suggested by the study of patients with fever described above, in whom the penicillin plasma clearance was 50 per cent greater than in the group of individuals observed under basal conditions.

The plasma clearance does not fall with the maximum plasma concentration of penicillin obtained in these experiments, which indicates that the upper limit of tubular excretion of this substance has not been reached. This was to have been expected since the normal renal tubules may excrete 50 mgm. of diodrast iodine per minute (5) and, at the maximum rates recorded here, less than 1 mgm. of pure penicillin per minute was being cleared.

Variations in the minute volume of urine have been shown here to have no effect on the rate of excretion of penicillin which is in accord with the observations of others using substances excreted by the renal tubules and in contrast to those in which such chemicals as urea and the sulfonamides were studied (5, 8).

Penicillin administered by the continuous intravenous route is quantitatively excreted by the kidney. No destruction in the tissues was demonstrated.

The optimal concentration of penicillin in the plasma and tissues for the treatment of various infections has not yet been determined but, in accord with the available evidence (9), it is probable that a plasma level of 0.20 to 0.30 units per

cc. is adequate to control severe staphylococcal infections and 0.05 to 0.1 units per cc. may be sufficient to cure infections caused by the hemolytic streptococcus, pneumococcus, and gonococcus.

By the intravenous route, 200,000 to 400,000 units per day should result in plasma levels satisfactory for the treatment of severe staphylococcal infections. Penicillin may be administered by continuous subcutaneous drip in such instances if intravenous therapy is impractical, but the observations presented here definitely indicate that much larger daily doses should be used. By either route, 100,000 to 200,000 units per day should be sufficient for the management of hemolytic streptococcus, pneumococcus, and gonococcus infections.

Only many further detailed studies will determine the optimal mode of administration and dosage of penicillin.

SUMMARY AND CONCLUSIONS

1. The absorption and excretion of penicillin during continuous intravenous and subcutaneous administration has been studied.

2. Predictable plasma levels were obtained, those following intravenous being higher than those following subcutaneous infusion.

3. Penicillin was removed from the plasma by the kidney at rates which indicate that the renal tubular excretory mechanism was principally involved in this process.

4. The relationship of these observations to the use of penicillin in the treatment of human infections is discussed.

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A PRINCIPLE FROM LIVER EFFECTIVE AGAINST SHOCK DUE TO BURNS[1]

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The purpose of this report is to describe a new method of evaluating therapeutic activity in shock and to present evidence for a principle in liver extract, which has the activity of significantly decreasing the mortality which follows a standardized scalding burn.

The development of new therapeutic agents useful in shock has, in large part, been impaired by the absence of standardized quantitative methods for evaluating anti-shock activity. During the past 2 years, a shock-producing procedure has been developed in this laboratory that has permitted satisfactory evaluation of anti-shock activity in the shock which occurs following a scalding burn. The shock-producing technic consists of immersing the entire body surface, except for the head and neck, of etherized rats and mice for definite intervals of time into a water-bath set at scalding temperatures. Therapeutic activity is evaluated by repetitive testing, where the percentage of survival and the average survival period of a group of treated animals is compared to suitable control groups, scalded and observed under identical conditions. This method allows objective control of the degree of thermal injury and thereby insures that the animals within groups are damaged to the same extent. Since the technic is simple and fast, the method permits the traumatization of large numbers of animals in any given experiment under identical conditions of room temperature, humidity, etc. By housing and feeding all rats or mice in any given experiment under identical laboratory conditions, factors such as the pre-experimental nutritional regime, tissue hydration, etc., are adequately controlled. Finally, the features which permit the employment of large groups of animals, allow the results obtained to be statistically evaluated for their significance. A similar scalding method has recently been described (1).

METHOD

The water-bath used in these experiments was a 10 gallon jug, in which the water was mixed by a stirring motor. The bath temperature was regulated by a thermostat so that it varied no more than ±0.5° C. After the bath had been set at the desired temperature, rats or mice were rapidly anesthetized with ether. The loose skin in the occipital region was then grasped with a hemostat, and the animal was plunged into the scalding water in such a manner that only the head and neck remained above water. After the desired interval of immersion, the animal was withdrawn and placed in a bucket containing sawdust to permit partial drying. The rate of traumatizing animals by this scalding procedure may be increased several-fold if animals are anesthetized in groups and 2 or more animals are simultaneously scalded. Using these procedures, 2 people may scald as many as 6 rats or mice every minute. The duration of exposure to the scalding temperature was generally 10 to 15 seconds. The accuracy of these immersion periods was approximately ±0.5 second. These intervals of exposure appeared to be optimal, since decrease in duration to 5 seconds decreased the accuracy of the exposure, while with the immersion periods above 20 seconds it was observed that animals tended to awaken from anesthesia and began to struggle. Ether anesthesia was used in preference to non-volatile anesthetics, such as avertin and nembutal, for the reason that we have found that avertinized and nembutalized animals which had been scalded awakened from anesthesia very slowly. Following the start of the experiment, food and water were withheld from the animals.

Survival observations after thermal injury were conducted over a 48-hour period, and all experiments were terminated at that time to prevent the effects of infection and other secondary disturbances, which later become operative, from influencing mortality. The groups were observed at regular intervals, the dead animals being removed and the time of death noted. A small percentage of the animals died within the first 30-minute period following scalding and these animals were discarded from the experimental groups because it seemed unlikely that death was due to secondary shock. All animals still alive when the experiment was terminated, were arbitrarily assigned a survival period of 48 hours.

Upon awakening from anesthesia, scalded animals show characteristic changes before dying. Immediately follow-

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ing scalding, there is an apparent recovery; however, increased irritability and decreased spontaneous activity are evident. Later, the skin becomes cold, dyspnea develops, the animals become profoundly asthenic, and eventually death occurs. A short time after immersion, the tail and extremities become edematous, and if the animal survives the first 24 hours, dry gangrene of the edematous peripheral extremities usually is evident.

PATHOLOGICAL CHANGES

Post mortem examination of animals dying in shock as the result of thermal injury revealed the following macroscopic findings: pulmonary congestion was regularly observed, visceral congestion was usually found, and hemorrhagic adrenals and gross hematuria were irregularly seen. Those findings which appeared seemed to be related to the period of survival following scalding, becoming more frequent as the survival time was increased. Thus, animals dying immediately or soon after scalding, while exhibiting pulmonary congestion, rarely showed visceral congestion, adrenal changes, or gross hematuria. However, as the period of survival increased, the frequency of visceral congestion, hematuria, and adrenal damage likewise progressively increased. Microscopic examination revealed congestion in lung, intestine, kidney, adrenals, and other organs.

EFFECT OF THERMAL INJURY ON BLOOD CONCENTRATION AND SKIN TEMPERATURE

We have found that a significant degree of hemoconcentration is consistently evident following scalding. It has been shown that mice which have been subjected to scalding likewise exhibit hemoconcentration (1). Figure 1 illustrates typical findings on changes in skin temperature following scalding. It will be seen that decrease in blood flow to the skin accompanies the clinical appearance of shock in animals subjected to a scalding burn.

INFLUENCE OF SEX, STRAIN, BODY WEIGHT

The rats and mice used in these studies, obtained from different breeders and representing various strains, were fed Purina fox chow and water *ad lib.* until the time of experimentation. Recognizing that animals from different strains might exhibit varying degrees of resistance to thermal injury, all experiments were designed in such a manner that animals of a given weight range from the same source were selected at random to form the various groups. The mice used in these studies weighed between 18 and 25 grams and the rats varied from 200 to 400 grams.

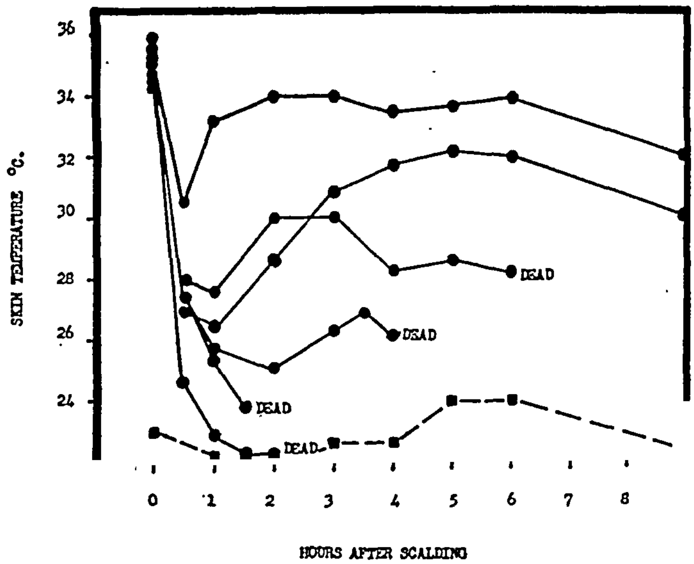

FIG. 1. THE EFFECT OF SCALDING MICE UPON SKIN TEMPERATURE

(●— ●) represents individual curves of mice showing representative examples; (■— ■) room temperature.

The variation of body weight of rats within groups in a single experiment, however, was no more than ±25 grams. It may be of interest to mention that with rats, and possibly mice, it appeared that smaller animals died more rapidly than larger animals following comparable thermal injury.

To check on the possible effects of sex difference upon resistance to thermal injury, animals derived from the same source and in the same weight range, were divided into groups on the basis of sex and scalded under identical conditions. The following data are from a representative experiment on this point: of 72 male mice scalded at 60° C. for 10 seconds, 36 per cent survived and the average survival time was 31.4 hours, while 54 females had a percentage of survival of 43 per cent, and the average survival time was 34.0 hours. These differences are not large, and as will be shown later, are not significant. Similar experiments with rats likewise have demonstrated that the sex of the animal has little influence upon the survival time following scalding.

EFFECT OF VARIATION OF IMMERSION DURATION AT VARIOUS SCALDING TEMPERATURES UPON THE SURVIVAL TIME OF RATS AND MICE

A priori, it would appear that the degree of thermal injury inflicted by scalding, as evidenced

by time mortality curves, should be a function of the duration of exposure and of the scalding temperature. It may be expected that if the thermal injury is too slight, an insufficient number of control animals would die and therapeutic activity would not be measurable. On the other hand, if the thermal injury be progressively increased, it might be expected that a point would be reached where the damaged animals would die rapidly, irrespective of treatment. It would therefore appear that a degree of trauma, intermediate between these extremes, would represent the desirable conditions for evaluating therapeutic activity. Experiments were performed to find the minimal degree of trauma which would kill most of our animals in a 48-hour period. Two experiments are shown in Figures 2a and 2b, one with rats, the other with mice, which illustrate the method of determining the optimal conditions of exposure and temperature for evaluating therapeutic activity. It is clear from these data that the rate of mortality is a function of the exposure duration and of the bath temperature, and may be adjusted to desired levels by alteration of the scalding conditions.

The conditions for scalding our animals which have generally proved satisfactory for evaluating therapeutic activity were an immersion duration of 10 seconds at 65° C. for mice weighing approximately 20 grams; and 10 or 15 seconds at 75° C. for rats weighing approximately 200 grams. It should be emphasized that the conditions which proved satisfactory for evaluating therapeutic activity in this laboratory may vary for other strains of animals or under different laboratory conditions.

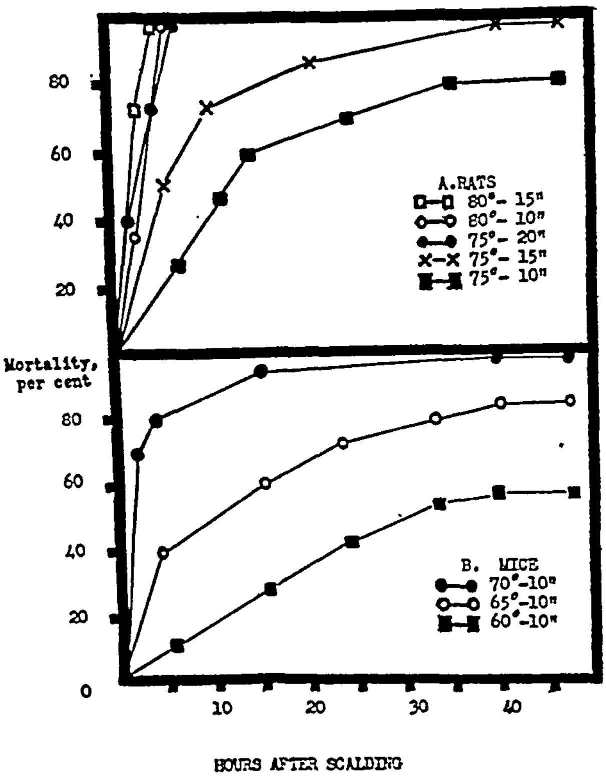

FIG. 2. SHOWS THE EFFECT OF VARYING THE SCALDING TEMPERATURE OR THE IMMERSION TIME UPON TIME MORTALITY CURVES

Each curve represents 50 animals. It will be seen that the higher the temperature or the longer the period of immersion, the greater the rate of mortality. The significance of these curves as regards absolute values is limited because only one experiment was performed.

VARIABILITY INHERENT IN THE SCALDING METHOD

The variations in response which exist with all shock-producing procedures has been emphasized (2). The scalding procedure described provides a statistical approach to the problem of overcoming the factor of individual animal variation in that the response of large groups of animals to standardized thermal injury is studied under identical conditions. To determine the variations inherent in the scalding technic, animals of the same strain and weight range were divided into 5 or more groups of 20 to 30 animals per group by random selection. The animals in each group received no treatment and were scalded and observed under identical conditions. The results obtained in 2 experiments using rats and in 2 experiments with mice are illustrated in Figures 3a, b, c, and d. It will be seen that considerable variation between presumably identical groups consistently occurs. Using either the average troup survival time or the percentage of survival of the groups as a criterion for the response to thermal injury, it is evident that the variability between the extremes may be as great as or greater than 100 per cent. Differences of this degree, using 20 or 30 animals per group obtained by random selection, can be calculated by statistical methods to be significant. This discrepancy between the actual and the expected degree of variability, indicates that there is some

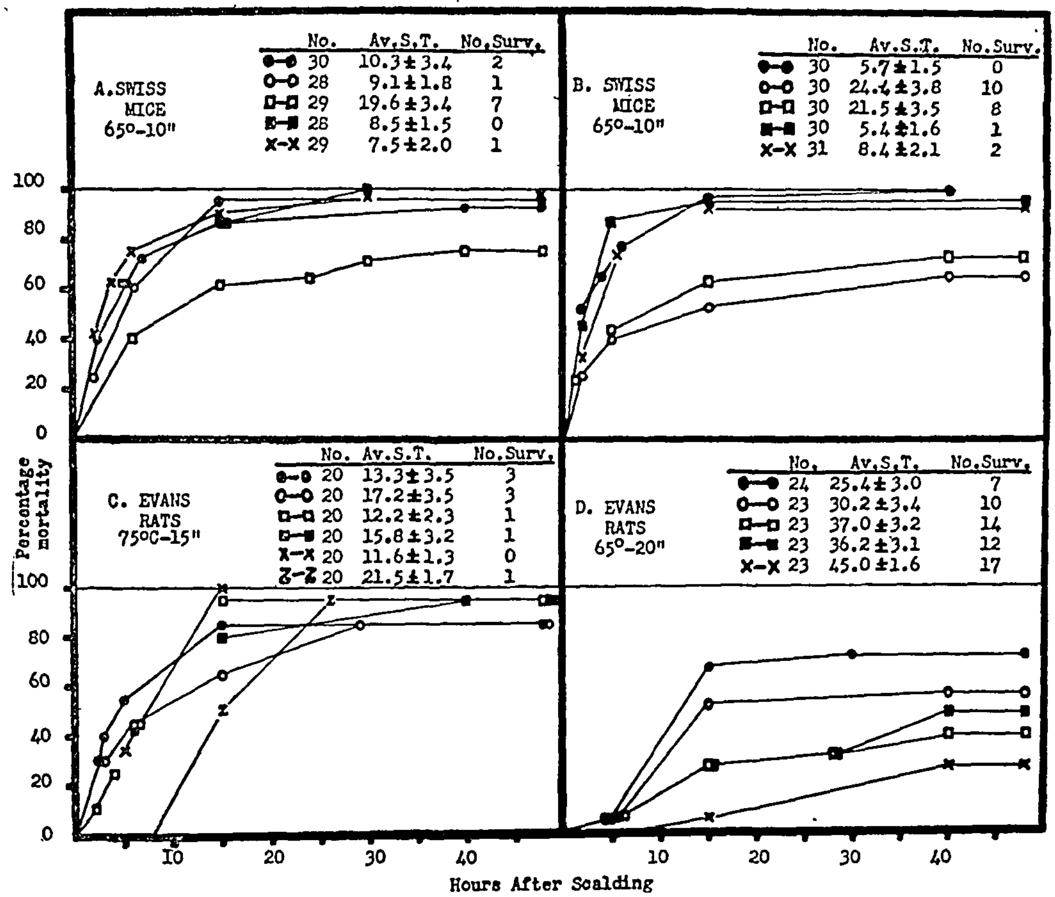

Fig. 3. Four Experiments, 2 with Mice, 2 with Rats, Which Illustrate the Variability of Groups of Untreated, Presumably Identical Animals, Scalded Under Identical Conditions

Each curve represents a group consisting of 20 to 31 animals. It may be seen that considerable variation exists both in the average survival time (Av. S. T.) and in the number surviving (No. Surv.). The standard error is shown following the Av. S. T., and it may be observed that these differences can be calculated to be statistically significant.

unknown variable operative in the experiment, which is capable of significantly modifying the response to standardized trauma.

Recognizing the limitations imposed by the variability inherent in the scalding procedure, it would appear that the evaluation of therapeutic activity is measurable only with the conditions of repeated testing. Let us consider the type of effects which would be obtained with active and inactive anti-shock agents:

(*a*) If a substance does not possess anti-shock activity, in a sufficient number of tests the results should depend only upon chance; thus, it is possible in a single trial to obtain positive results, which appear to be statistically significant, using as many as 30 animals to a group. Likewise, apparently deleterious effects may be obtained with inert material. In many instances, the control and treated groups will be identical. This may serve to explain why statistically significant positive therapeutic effects can be obtained with an agent possessing no anti-shock activity, and is possibly an explanation for certain differences of opinion regarding the anti-shock activity of certain agents. (*b*) If the agent under test has the activity of increasing average survival time only about 100 per cent, which is approximately the range of variation, on repeated testing, definite positive effects should be obtained in some cases; in other tests, the treated group should show the same response as the control groups, but in no instances should the agent be worse than the controls. (*c*) If the agent has powerful anti-shock activity (assume

TABLE I

Effect of treatment with 15 unit liver on the average survival time and percentage survival of mice subjected to scalding, arranged according to survival time of the control groups

Controls				Liver				
Exp. No.	Number mice	Average survival time	Number survived	Number mice	Average survival time	Number survived	Increase in average survival time	Increase in survived
		hours			*hours*		*per cent*	*per cent*
AVERAGE SURVIVAL TIME CONTROL GROUPS LESS THAN 5 HOURS								
1	26	2.3	0	32	13.1	0	+470	0
2	41	3.8	0	36	4.7	2	+ 23	+5.5
3	36	4.1	0	31	3.7	0	− 9	0
4	32	4.1	0	33	10.7	3	+161	+9.1
AVERAGE SURVIVAL TIME CONTROL GROUPS 5.0 TO 13.0 HOURS								
5	33	5.2	0	27	27.5	6	+430	+22
6	30	5.7	0	29	14.8	13	+160	+45
7	30	6.6	2	28	9.2	3	+ 39	+ 4
8	24	7.0	0	23	16.4	11	+134	+48
9	31	7.7	0	28	5.4	0	− 30	0
10	19	8.0	1	25	29.6	10	+270	+35
11	23	8.3	1	25	21.9	7	+158	+24
12	33	8.6	3	34	25.9	14	+201	+32
13	37	8.7	0	40	39.8	25	+358	+63
14	25	9.4	4	29	20.0	22	+113	+60
15	34	10.8	3	34	21.9	11	+103	+23
16	27	11.4	0	25	28.2	11	+147	+44
17	27	11.5	0	30	33.7	17	+193	+57
18	36	11.9	1	35	16.5	6	+ 39	+14
19	20	12.5	0	20	42.0	15	+236	+75
20	30	12.5	4	28	29.6	9	+137	+19
AVERAGE SURVIVAL TIME CONTROL GROUPS 13 TO 20 HOURS								
21	21	13.4	0	20	25.0	3	+ 87	+15
22	16	14.3	0	19	25.0	5	+ 75	+26
23	27	14.9	0	28	23.2	0	+ 36	0
24	41	15.2	2	40	33.0	14	+117	+30
25	29	15.6	3	30	26.6	10	+ 70	+23
26	35	16.3	5	33	28.0	16	+ 42	+39
27	33	18.2	7	35	21.3	11	+ 17	+10
28	40	19.7	6	38	33.2	18	+ 69	+32
29	33	19.6	10	32	30.6	16	+ 36	+20
30	40	19.9	8	39	27.4	19	+ 38	+29
AVERAGE SURVIVAL TIME CONTROL GROUPS ABOVE 20 HOURS								
31	30	21.0	8	30	27.9	14	+ 42	+20
32	42	21.8	12	39	32.4	13	+ 49	+ 6
33	27	22.0	4	30	39.0	19	+ 77	+48
34	39	23.7	7	38	28.8	13	+ 21	+16
35	44	24.0	16	39	32.0	19	+ 33	+13
36	36	31.0	15	33	29.5	15	− 5	+ 4
37	29	38.0	20	31	37.4	18	− 2	−10
38	30	39.1	16	34	35.1	17	− 11	− 3
39	30	42.6	24	30	43.7	26	+ 3	+ 7

that it increases the average survival time 500 per cent), this activity would be consistently evident despite a variation of 100 per cent.

RESULTS

Effect of liver extract

In the course of preliminary testing, it was observed that 3 commercial liver extracts, which contain anti-anemia activity, all appeared to increase the survival time of animals subjected to scalding. Extensive testing of one of these extracts was undertaken to establish whether or not this action of liver extract was reproducible and significant. In these experiments, mice were the test animals; the liver extract (Lederle), containing 15 injectable U.S.P. units of anti-anemia activity per ml. in 0.5 per cent phenol, was injected intraperitoneally in a dosage of 1.0 ml. per 100 grams body weight, approximately 0.5 hour prior to scalding. As a control for the fluid administered, groups of mice were injected with equivalent amounts of either physiological saline or 0.5 per cent phenol, at the same time interval prior to experimentation. In some experiments, a second control group, which received no treatment, was used in addition to the fluid control.

Table I illustrates the results of 39 experiments, using 2,426 mice, where the effect of liver on the average survival time and on the percentage survival is shown. The table has been subdivided arbitrarily into 4 divisions corresponding to the degree of thermal injury as evaluated by the average survival time of the control group. Figure 4a shows the effect of liver treatment upon the survival time and upon the percentage survival, at these varying ranges of thermal injury. It will be seen that liver has a tendency to increase the average survival time and to decrease the percentage mortality under all conditions of thermal injury. However, it is clear that this anti-burn shock activity of liver is best evident when the conditions of thermal injury are such that the control groups die with an average survival time varying from 5.0 to 20.0 hours, since the activity decreases when the conditions of thermal injury are either decreased or increased so that the significance of the effects at both extremes is questionable.

Figure 4b shows that the control solutions, saline and 0.5 per cent phenol, given in amounts

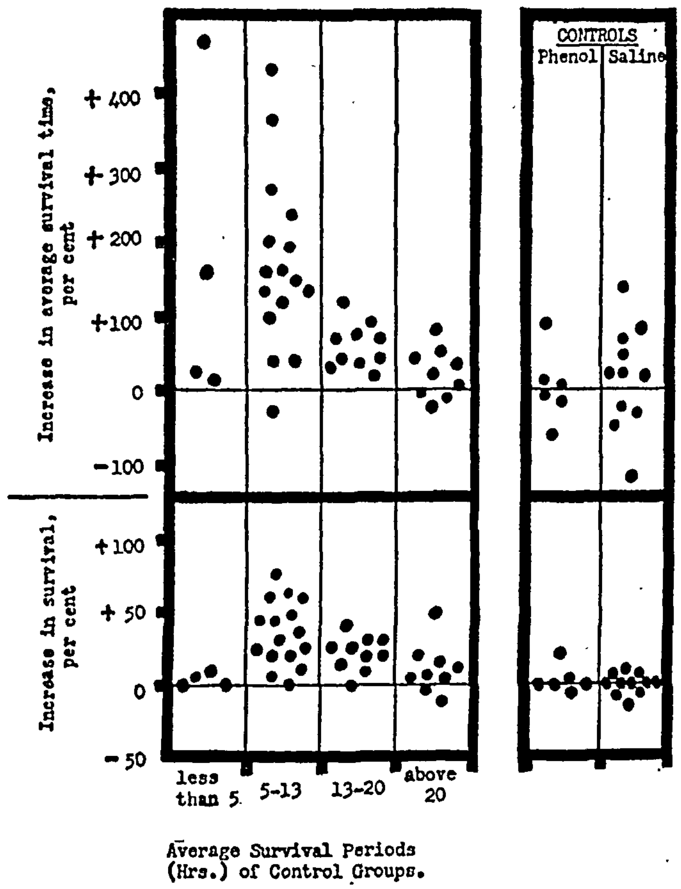

FIG. 4A. THE EFFECT OF 15 UNIT LIVER ON THE AVERAGE SURVIVAL TIME AND ON THE PERCENTAGE SURVIVING AS COMPARED TO THE FLUID CONTROL, AT VARIOUS RANGES OF THERMAL INJURY

Each dot represents the response of a group comprising 19 to 40 animals, the total number of mice employed in this study being 2426 mice. It can be seen that in ranges where the control average survival periods fell between 5 and 13, and 13 and 20 hours, that liver treatment increased the percentage surviving and the mean survival time in most instances. However, the anti-shock activity of liver is not marked, if the degree of thermal injury is too great or too slight.

FIG. 4B. THE EFFECT OF THE CONTROL SOLUTIONS, SALINE AND 0.5 PER CENT PHENOL, AS COMPARED TO NO TREATMENT

It will be seen that either solution is an adequate control. These data demonstrate, as does Fig. 4a, the range of variations of control groups.

equivalent to 1 per cent of the body weight, 0.5 hour prior to trauma, had no significant effect and it is further evident that either solution would serve equally as well as a fluid control for liver extract.

The question naturally arises as to why liver shows significant activity in certain ranges of thermal injury, and is without marked effect when the degree of trauma is increased or de-

creased. As has been previously mentioned, it was predicted that there would be conditions of thermal injury which are unsatisfactory for evaluation of anti-shock activity. Thus, when the degree of trauma is too great, it was mentioned that the animals may die rapidly irrespective of treatment and this situation appears to have occurred in those experiments where the average survival time of the controls was less than 5 hours. Under conditions wherein the degree of trauma was relatively slight, the relative ineffectiveness of liver may be due to the fact that an insufficient number of control animals died, or it may be that the *in vivo* concentration of the liver principle becomes depleted early in the course of the experiment so that it cannot effectively combat the deaths that occur later.

Effect of 5 per cent saline and of liver extract plus 5 per cent saline

A. Effect of large volumes of saline

As early as 1926, it was observed that the chloride of whole blood and plasma was significantly lowered following burns (3), and large amounts of saline were administered as one aspect of the treatment of burns with good results (4). We have found that the intraperitoneal administration of 0.9 per cent sodium chloride given, in amounts equivalent to 5 or 10 per cent of the body weight, to mice and rats after scalding, significantly decreased mortality. This effect has been independently observed (1) and it was shown that the sodium salts of various acids, given in isomolar concentrations, are as effective as sodium chloride solutions in preventing death following a scalding burn (5). It was further found in this laboratory that saline given in amount equivalent to 5 per cent of the body weight 0.5 hour prior to scalding decreased mortality. (Table II).

B. Effect of liver plus saline as compared to saline alone

It was of interest to determine whether combined pretreatment with liver plus saline was superior to pretreatment with salt solution alone. Using mice, 3 experiments were performed in the following manner: Three groups containing at least 30 mice per group received one of the following treatments; (*a*) physiological saline, 5 per cent of the body weight, administered intraperitoneally approximately 0.5 hour prior to trauma; (*b*) 15 unit liver extract in saline, intraperitoneally, 0.5 hour before scalding, in amounts so that the dosage of liver extract was 1.0 ml. per 100 grams of body weight and the volume of salt solution was 5 per cent of the body weight; and (*c*) a control, receiving no treatment. The composite results of the 3 experiments are shown graphically in Figure 5. It will be seen that saline increased the survival time 238 per cent and the percentage surviving 22.3 per cent over the control non-treated group. Liver plus saline treatment increased the average survival time 352 per cent and the percentage surviving 35.8 per cent over the non-treated control group. This represents an increase of 144 per cent in

TABLE II

The effect of 5 ml. saline per 100 grams of body weight, given intraperitoneally 0.5 hour prior to scalding, in mice and rats at 65° for 10 seconds

Test animals	Exp. No.	No treatment			Saline			Increase in survival time	Increase in survived
		Number animals	Average survival time	Number survived	Number animals	Average survival time	Number survived		
			hours			*hours*		*per cent*	*per cent*
Mice	1	25	5.7	0	20	12.5	1	+120	+ 5
	2	22	7.2	0	22	7.5	0	+ 4	0
	3	20	14.8	2	20	35.8	5	+142	+15
	4	21	8.3	1	21	17.3	4	+108	+15
	5	66	9.3	4	64	15.3	10	+ 65	+10
	6	50	4.9	0	48	27.7	19	+455	+40
	7	30	2.9	0	29	10.8	5	+272	+17
Rats	8	30	24.0	7	29	38.6	19	+ 61	+43
	9	35	3.2	0	35	7.0	1	+119	+ 3

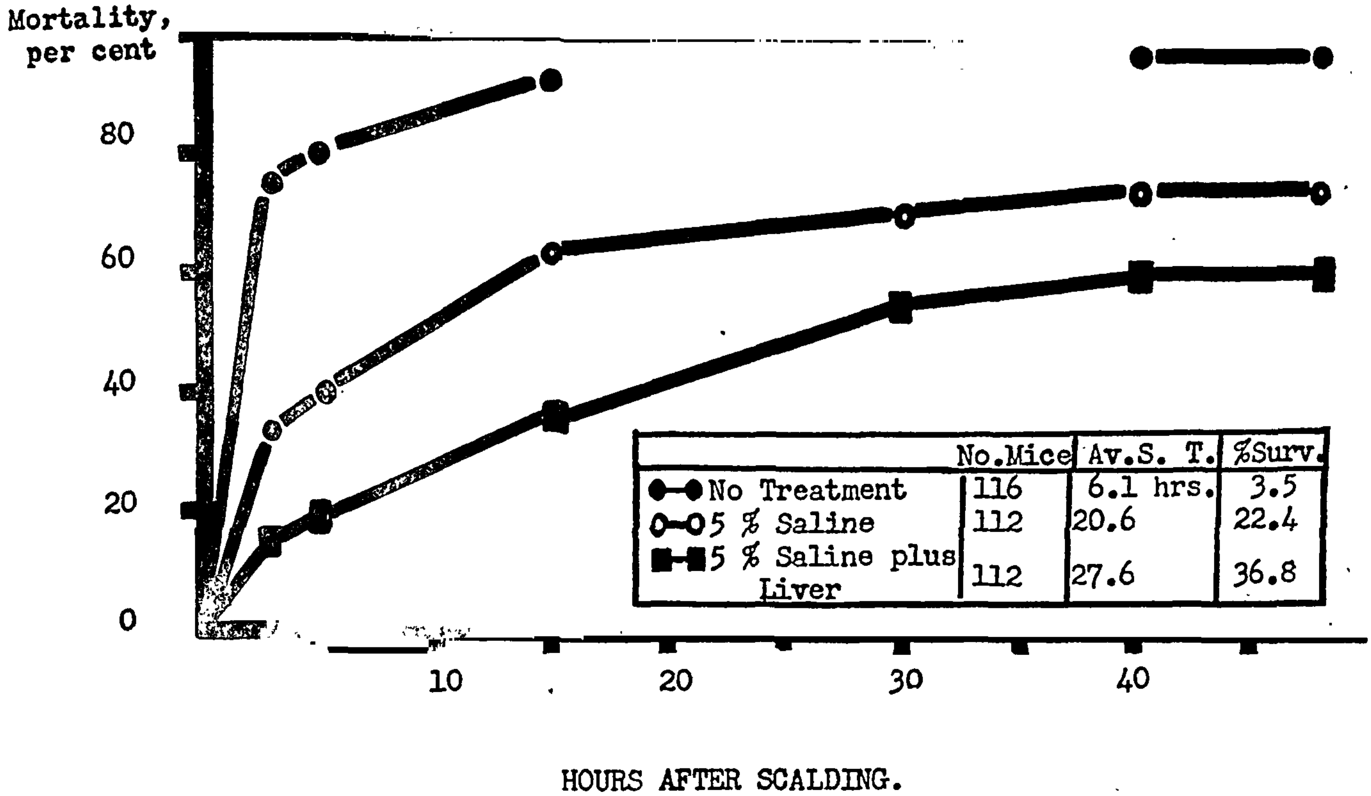

FIG. 5. THE EFFECT OF LIVER PLUS 5 PER CENT SALINE AND OF 5 PER CENT SALINE ALONE UPON MICE SUBJECTED TO SCALDING AT 70° C. FOR 10″

Each curve represents the composite results of 3 experiments, using a total of 112 to 116 animals per group. These data show that liver plus saline is superior to saline alone.

average survival time and a 13 per cent increase in the percentage of survival for the combined treatment. In these 3 experiments, the individual percentage differences in average survival time between liver plus saline as compared to saline alone over non-treated controls, was +260 per cent, +100 per cent, and +92 per cent, respectively.

Using rats as the test animals, 9 experiments of the following type were performed: rats were divided into 2 groups containing at least 15 animals per group; one group received liver extract subcutaneously 18 hours prior to experimentation, and at approximately 0.5 hour prior to scalding, both groups were intraperitoneally injected with 0.9 per cent NaCl, in amounts equivalent to 5 per cent of the body weight and were then scalded under identical conditions. In these experiments, Lederle 15 unit liver extract was tested in 4 experiments, and in the 5 remaining tests, a Lilly liver extract containing 2 U.S.P. anti-anemia units per ml. was employed. The dosage of liver extract used was 0.7 ml. per 100 grams of body weight for the 15 unit liver, and 0.8 ml. per 100 grams of body weight for the 2 unit liver. The results obtained are illustrated in Figure 6. It will be seen that in 9 trials, where the average survival time of the saline controls was less than 15 hours, liver plus saline treatment was consistently superior to saline alone.

Is the anti-shock activity identical with the anti-anemia principle?

The 15 unit liver extract, which has been shown in the previous sections to possess anti-shock activity contains the liver anti-anemia principle. It was, therefore, of interest to determine whether or not the activity against burn shock was due to the anti-anemia activity present in the extract. To answer this question, the anti-shock activity of liver extracts containing anti-anemia activity in a high degree of purification was determined. These purified anti-anemia extracts furnished us by Dr. Y. Subbarow of the Lederle Laboratories were prepared by methods previously described (6) which may be briefly summarized as follows: The activity in 15 unit liver was precipitated as

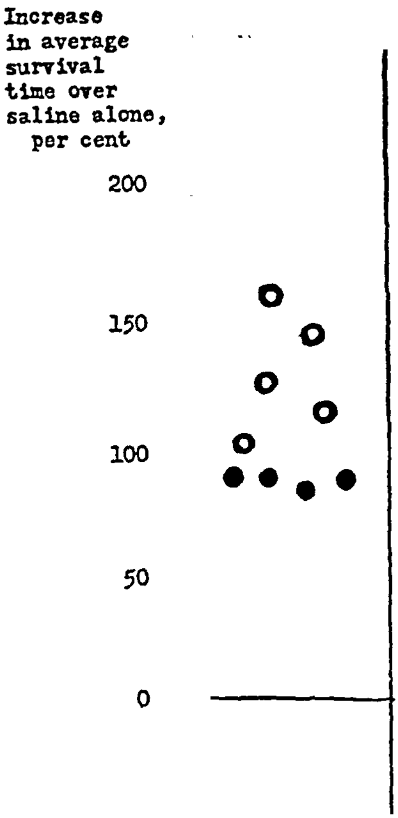

FIG. 6. THE EFFECT OF LIVER PLUS SALINE AS COMPARED TO SALINE ALONE IN RATS SCALDED AT 85° FOR 15 SECONDS

Circles represent 2 unit liver extract; black dots, 15 unit liver. Each dot or circle represents 15 to 20 rats.

a Reinecke salt; the reagent was removed; the anti-anemia activity was absorbed by charcoal and subsequently eluted. At this stage, the material, designated in this discussion as the charcoal absorbant, contains one U.S.P. anti-anemia unit per mgm. On treatment of the charcoal absorbant with 10 volumes of 1:1 acetone-ether, a precipitate, containing all of the anti-anemia activity, is obtained.

The anti-shock activity of both extracts containing an amount of anti-anemia principle equivalent to 15 unit liver, was evaluated by comparing the survival of groups injected with these fractions, with the results obtained with 15 unit liver and a saline control simultaneously performed. Seven experiments of this type were performed and the results are shown in Figure 7, where the percentage increase in average survival time over the control produced by 15 unit liver and the purified extracts is illustrated. It will be seen that while 15 unit liver is active, the purified extracts are consistently without significant activity. These data demonstrate that the anti-shock activity present in 15 unit liver is removed from the liver extracts by procedures which are used to purify anti-anemia activity. It is therefore concluded that the activity against burn shock present in liver extracts is not the anti-anemia principle.

Solubility properties of the principle effective against burn shock

In collaboration with Drs. Y. Subbarow and N. Bohonos of the Lederle Laboratories, we are attempting to isolate the anti-burn shock principle in liver. Fractions of 15 unit liver prepared by Drs. Subbarow and Bohonos were tested in this laboratory in the manner described previ-

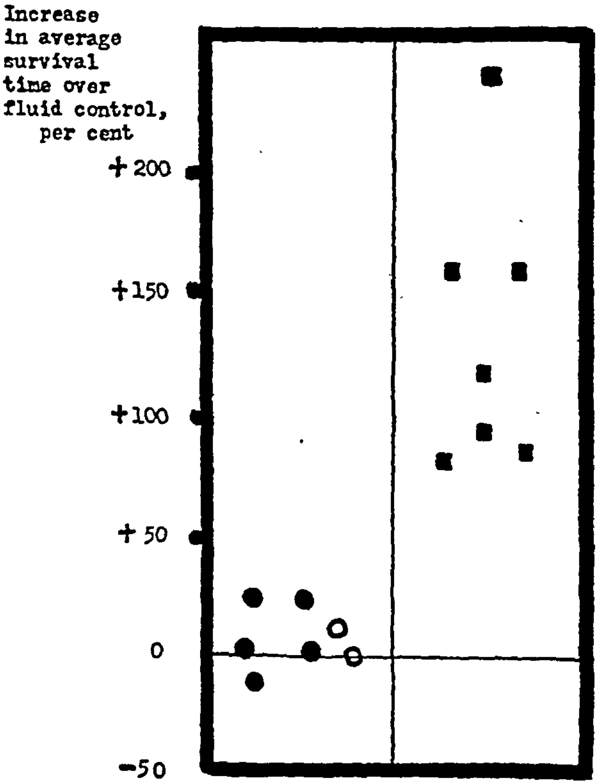

FIG. 7. THE ANTI-SHOCK ACTIVITY OF PURIFIED ANTI-ANEMIA LIVER EXTRACTS AS COMPARED TO 15 UNIT LIVER EXTRACT

Dots, charcoal absorbant; o circles, acetone-ether precipitate of charcoal absorbant; black squares, 15 unit liver. It may be seen that neither of the purified anti-anemia extracts possess anti-shock activity.

ously for anti-anemia extracts. Figure 8 is a flow sheet which describes the chemical procedures employed to obtain the various fractions. Figure 9 illustrates the percentage increase in the average survival time over the fluid control group, produced by treatment with these fractions as compared to the 15 unit standard concurrently tested. The results may be summarized as follows: (*a*) On treatment of 15 unit liver with acetone, the activity is precipitated, little or no activity remaining in solution. (*b*) On treating this acetone precipitable fraction with ethanol, a precipitate is obtained which is active but possibly contains less activity than 15 unit standard. The ethanol solution appears to be inactive, but may contain slight activity. (*c*) Treatment of the acetone precipitable fraction of 15 unit liver with methanol gives rise to a precipitate which appears inactive as compared to the standard; however, slight activity may be present. The methanol solution likewise appears relatively inactive, but further testing would seem to be necessary before final conclusions are drawn on this point. (*d*) The absorbed fraction obtained by treatment of 15 unit liver with superfiltrol is active, although somewhat

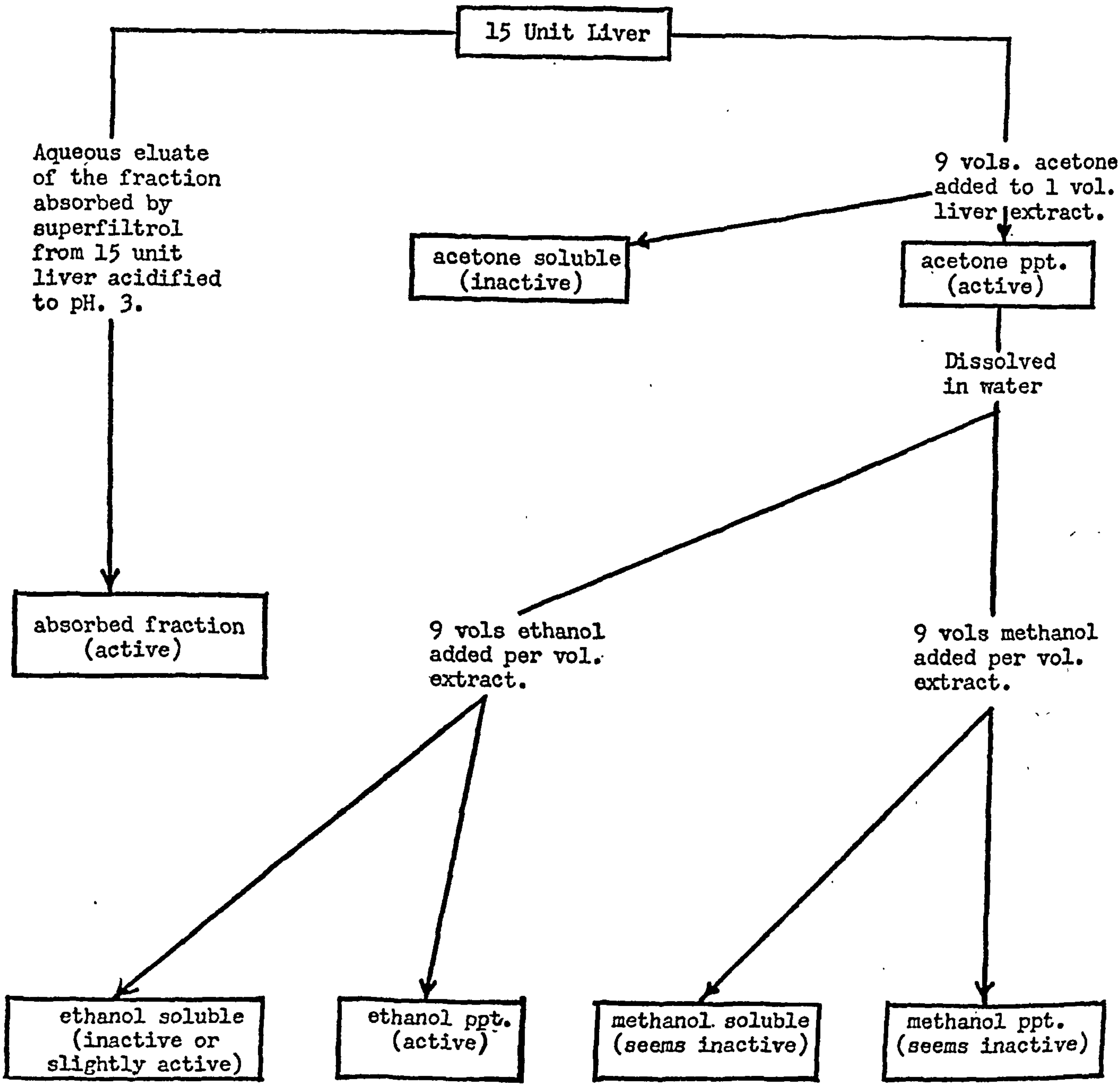

FIG. 8

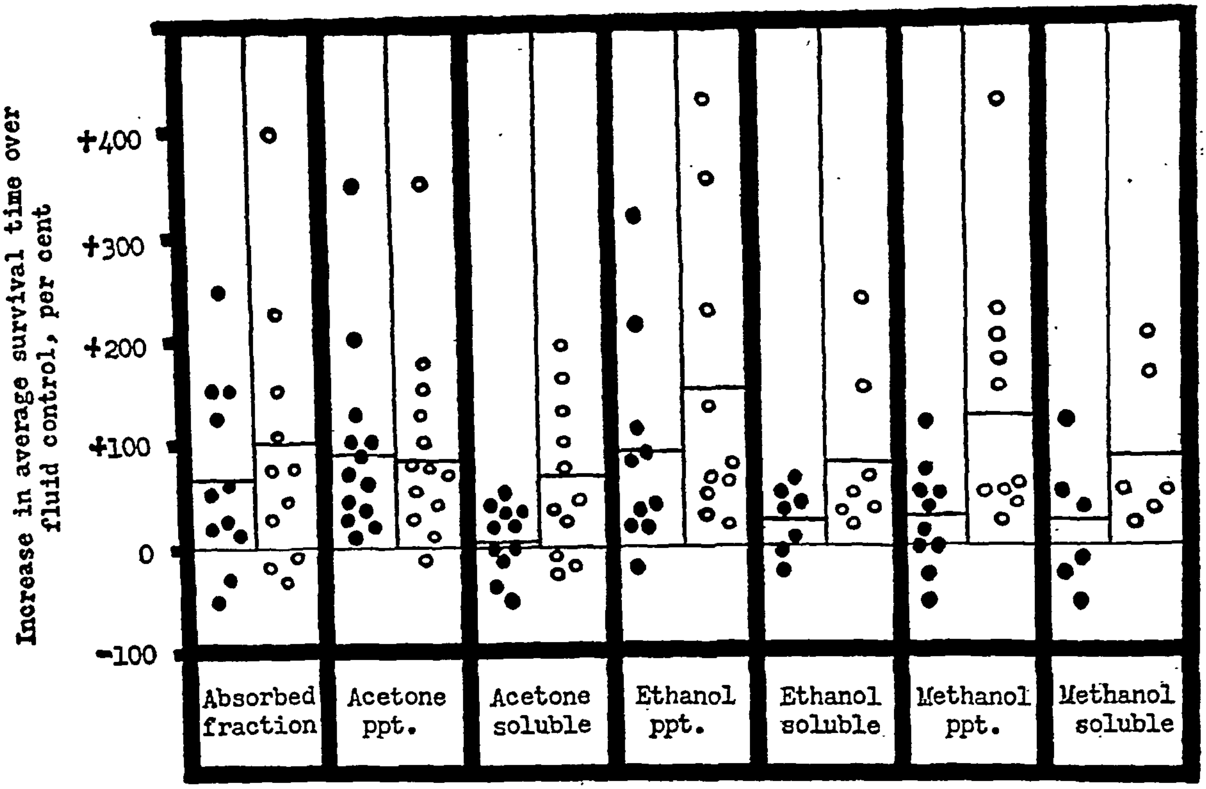

FIG. 9. ANTI-BURN SHOCK ACTIVITY OF VARIOUS FRACTIONS DERIVED FROM 15 UNIT LIVER EXTRACT
The black dots represent the fraction, the circles show the response with 15 unit liver. The cross lines represent the average of the responses. Each dot or circle is an individual experiment comprising 19 to 40 mice per group.

less than that obtained with the 15 unit standard. This difference, however, is not large and may not be significant. Discussion of the chemical nature of the anti-burn shock factor would appear to be premature, further work being necessary before the chemical nature of the activity can be characterized.

DISCUSSION

The evidence presented demonstrates the existence of an anti-burn shock activity in liver extracts. The mechanism by which the liver factor acts is as yet unknown, but it is possible that the liver principle either compensates for the fluid loss associated with burns, inhibits the activity of toxic substances believed by some to play a rôle in the etiology of shock, or corrects some other factor important for the production of shock. It will have been noted that the activity of liver extract against burn shock was evident when the material was given prior to trauma. In terms of practical usefulness, it is important to know whether liver extract is effective when administered after the onset of shock. In this connection, we have found that the intraperitoneal injection of 15 unit liver at various time intervals after trauma while occasionally exhibiting activity, nevertheless appeared to be without significant effect. The finding that liver extract administered after scalding is usually ineffective in modifying survival, may possibly be due to the fact that large amounts of extraneous tissue constituents are present in all types of commercial liver extracts. It is likely that such impurities are deleterious to shocked animals, and thus may mask the therapeutic effects of the anti-shock activity. For this reason, experiments to determine the activity of liver extract administered after the onset of shock will be repeated when purified extracts of liver anti-burn shock activity are obtained. Questions related to dosage, route of administration, etc., have likewise been deferred until complicating constituents accompanying the anti-shock activity have been removed from the liver extracts.

Thus far, it has only been shown that liver is effective against burn shock when given prior to thermal injury and whether it may prove to be useful in other types of shock has yet to be determined. We have found that liver appears to be ineffective in rats subjected to tourniquet shock or acute hemorrhage and acute septicemia in mice. Details of these studies will be subsequently published.

The significant variation between large groups of apparently homogeneous animals is a characteristic of the scalding method which warrants special mention, since it may be expected that this feature is common to all methods of evaluating anti-shock activity. While it was recognized that extreme individual variation might be expected in response to standardized thermal injury, it was thought that variations could be reduced to a minimum by using group response to identical thermal injury, where variable factors such as anesthesia, environmental conditions, sex, body weight, strain, nutritional status, and hydration, were adequately controlled. This expectation of insignificant variation with groups of 20 to 30 animals was not achieved, thus it was necessary to evaluate anti-shock activity on the basis of repetitive testing. In a number of instances, we have encountered substances which on repeated assay proved to be inactive, but which in the preliminary tests (using as many as 60 animals) seemed to possess significant anti-shock activity. It is clear that the attainment of an apparently significant response to a particular treatment in shock does not warrant the assumption that the same response will necessarily be obtained in the future. The question naturally arises as to whether the degree of variability encountered with the scalding technic applies to other methods of evaluating anti-shock activity. It would appear reasonable to suppose that methods which do not allow the employment of large groups of homogeneous animals under identical conditions, would at least have the variability encountered with the scalding method, and most likely greater variability might be expected. This might indicate that the significance of results in shock therapy, which have not been repeatedly retested for consistency, is questionable, despite the fact that apparently significant responses were obtained in initial trials. It may well be, that controversial reports concerning the anti-shock activity of certain agents are, in part, due to a lack of appreciation of the degree of variability inherent in shock-producing procedures.

SUMMARY

1. A new method for evaluating anti-burn shock activity is described, wherein activity is measured by studying the response of groups of animals on repetitive testing.

2. It has been demonstrated that statistically significant positive therapeutic effects may be obtained with inert materials having no anti-shock activity when as many as 30 animals are used in both the treated and control groups. This offers an explanation for the differences of opinion regarding the anti-shock activity of certain agents.

3. There is a principle in certain liver extracts which possesses the power of decreasing the mortality and increasing the survival time of animals subjected to burn shock.

4. This anti-burn shock factor, present in some commercial liver extracts, is not the antipernicious anemia principle.

5. Some of the solubility properties of the anti-burn shock factor are described.

6. Intraperitoneal injection of 5 per cent of the body weight of saline is effective against shock.

7. Pre-treatment with liver extract plus saline is significantly more effective than salt solution alone.

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ELECTROLYTE AND FLUID STUDIES DURING WATER DEPRIVATION AND STARVATION IN HUMAN SUBJECTS, AND THE EFFECT OF INGESTION OF FISH, OF CARBOHYDRATE, AND OF SALT SOLUTIONS [1,2]

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Human volunteers were deprived partially, or completely, of water and food for several days. Balances of water, of nitrogen, and of various electrolytes were measured, together with the concentrations of these same substances in serum. From these data, the partition of the total water loss between extracellular and intracellular phases was estimated.[3] The possible therapeutic values of water, various salt solutions, carbohydrate, and whole fish were assayed in certain experiments.

CHEMICAL METHODS

Samples of serum and defibrinated whole venous blood were collected anaerobically. Concentrations of sodium, potassium, and chloride in serum were determined by the methods of Hald (1, 2). Carbon dioxide content of serum or plasma was measured by the method of Van Slyke and Neill (3), heparinized plasma equilibrated with carbon dioxide at a partial pressure of 40 mm. Hg being used in Experiment I. Total and non-protein nitrogen of serum and of whole blood were measured by the Kjeldahl technic. Water content of serum and of cells was calculated from the respective concentrations of protein (4). Volume of cells in whole blood defibrinated over mercury (5) was measured by means of Daland microhematocrit tubes.

In urine, chloride was determined by the Volhard-Harvey titration (2), sodium by the method of Butler and Tuthill (6), potassium by the method of Hald (1), and total nitrogen by macro-Kjeldahl.

Several fillets of fish (haddock) were analyzed for nitrogen, water, chloride, sodium, and potassium. Average values were used in calculating metabolic balances. Per kgm. of whole fish, these were: nitrogen 28.4 grams; water, 818 grams; chloride, 26.1 m.eq.; sodium, 29.0 m.eq.; and potassium, 80.6 m.eq.

METHODS OF CALCULATION

The rationale of the methods for measurement of total water, and extracellular and intracellular phases has been fully discussed elsewhere (7). The same methods of calculation were used in these experiments with a few modifications. The initial extracellular volume, E_I, was taken to equal 20 per cent of the body weight, and changes in this volume were calculated from changes in the balance and concentration of chloride alone. Insensible weight loss, IL, was taken as the change in weight plus the weight of ingesta minus the weight of excreta and blood samples. As IL so measured in the sweating subjects included sweat, the usual relationship between insensible weight loss and total metabolism was altered. In the calculation of ΔW in these experiments, the average IL of the other 3 control nonsweating subjects was substituted for the observed IL. This procedure assumes that the average total caloric expenditure of the sweating subjects was the same as that of the control subjects, and is justified by their similarity in height, weight, and activity. The difference between the observed IL of the sweating subjects and the average of the controls was assumed to represent sweat which contained 30 m.eq. of chloride per liter (8); this was included in the chloride balance. The somewhat arbitrary character of this procedure makes the calculation of ΔW, ΔE, and ΔI_I in the sweating experiments less certain than in other experiments.

EXPERIMENTAL PROCEDURE

The volunteers were healthy males, active members of the Army of the United States. Each experimental period

[1] Lt. Col. D. B. Dill, Q.M.C., A.U.S., was responsible for the inception of this work. Capt. J. M. Quashnock, M.C., A.U.S., assisted in the management of the experiments and in the analysis of the data, as well as serving as a volunteer subject. Capt. Wilson, A.C., A.U.S., Lt. Holmes, Sn.C., A.U.S., Lt. Chovnick, A.C. A.U.S. Sgts. Tressler and Roberts, and Pvts. Perzanowski, Leyzorek and McHugh, all of the A.C., A.U.S., served as volunteer subjects. Mrs. T. Mintz did much of the work of chemical analysis, assisted by other members of the laboratory staffs at Wright Field and at Yale. Dr. A. J. Eisenman made all observations dealing with changes in blood cells. Miss Barbara Russell of the Yale University School of Nursing and Dr. S. C. Harvey of the Department of Surgery provided facilities for the care of the subjects. The work was carried out in connection with an Army Air Corps Contract.

[2] This article has been cleared for publication by the War Department Bureau of Public Relations. The opinions expressed are those of the authors and do not necessarily reflect official views of the War Department.

[3] Changes in the concentrations of lipids in serum and of ketones in whole blood were also followed. These will be reported in another paper (9).

TABLE I

Plan of experiments

Experiment	Period days	Food: None	Food: Carbohydrate	Water: None	Water: Limited	Water: Unlimited	Other fluids
I, L	0 to 5	+			+		
F	0 to 5	+			+		
Q	0 to 4	+			+		
	4 to 5	+				+	
D	0 to 4	+			+		
	4 to 5	+					Dil. seawater[3]
P	0 to 5		+		+		
R	0 to 5		+		+		
W	0 to 5		+		+		
H	0 to 4		+		+		
	4 to 5		+				Dil. seawater[4]
IIA, Q[1]	0 to 3	+		+			
	3 to 4		+			+	
C[1]	0 to 3	+		+			
	3 to 4		+			+	
H	0 to 3	+		+			
	3 to 4		+			+	
T	0 to 3	+		+			
	3 to 4		+			+	

Experiment	Period days	Food: None	Food: Carbohydrate	Food: Fish	Water: None	Water: Limited	Water: Unlimited	Other fluids
IIA, R	0 to 3	+			+			
	3 to 4		+				+	
IIB, Q[1]	0 to 3		+	+				A. F. J.[2]
C[1]	0 to 3			+				A. F. J.[2]
H	0 to 3			+				A. F. J.[2]
T	0 to 3	+						A. F. J.[2]
R	0 to 3	+						0.6 per cent NaCl
III, H	0 to 2	+				+		
	2 to 6			+	+			
	6 to 7		+				+	
C	0 to 2	+				+		
	2 to 6	+			+			
	6 to 7		+				+	
T	0 to 2	+				+		
	2 to 4	+			+			
	4 to 5	+					+	
M	0 to 2	+				+		
	2 to 4			+	(+)			
	4 to 5	+					+	

[1] Exposure in hot room.
[2] Limited amounts of "artificial fish juice."
[3] Solution containing 1 part of seawater to 3.5 parts of fresh water.
[4] Solution containing 1 part of seawater to 5 parts of fresh water.

TABLE IIa

Balance of water, electrolytes, and nitrogen

Experiment I

Expt.	Period	Intake: Oral H_2O	Output[1]: Urine Vol.	Urine Na	Urine Cl	Urine K	Urine N
	days	*cc.*	*cc.*	*m. eq.*	*m. eq.*	*m. eq.*	*grams*
I, L	0 to 1	170	865	130	177	74	8.9
	1 to 2	375	428	60	59	30	9.0
	2 to 3	500	483	47	31	40	12.9
	3 to 4	500	468	18	16	32	12.9
	4 to 5	500	433	6	2	32	12.2
I, F	0 to 1	375	450	41	71	69	8.3
	1 to 2	500	480	39	32	31	10.9
	2 to 3	500	690	42	21	35	15.9
	3 to 4	500	680	20	15	35	12.0
	4 to 5	1000	795	26	11	60	13.4
I, Q	0 to 1	0	605	114	86	53	8.6
	1 to 2	0	403	62	69	28	7.2
	2 to 3	180	298	29	26	26	6.5
	3 to 4	225	361	54	46	27	7.3
	4 to 5	2080	630	38	22	34	9.9
I, D	0 to 1	150	720	111	157	76	6.5
	1 to 2	300	585	86	78	38	8.6
	2 to 3	325	535	42	26	42	10.9
	3 to 4	425	735	68	20	49	13.7
	4 to 5	2800[2]	945	111	69	71	13.9

Expt.	Period	Intake: Oral H_2O	Intake: Oral Carbohydrate	Output: Urine Vol.	Urine Na	Urine Cl	Urine K	Urine N
	days	*cc.*	*grams*	*cc.*	*m. eq.*	*m. eq.*	*m. eq.*	*grams*
I, P	0 to 1	330	100	330	55	53	26	6.1
	1 to 2	500	100	180	16	12	7	4.2
	2 to 3	500	100	275	17	16	15	6.2
	3 to 4	500	100	155	2	0	7	3.7
	4 to 5	500	100	35	1	0	2	0.7
I, R	0 to 1	140	100	415	79	71	32	7.3
	1 to 2	320	100	258	31	43	12	5.3
	2 to 3	500	100	165	6	0	12	3.7
	3 to 4	500	100	151	3	4	8	3.3
	4 to 5	500	100	238	4	4	21	5.6
I, W	0 to 1	425	100	570	92	105	38	10.1
	1 to 2	500	100	415	51	54	23	8.4
	2 to 3	500	100	307	19	24	21	6.5
	3 to 4	500	100	312	19	20	20	7.2
	4 to 5	500	100	295	12	12	28	7.4
I, H	0 to 1	290	100	720	206	213	59	11.7
	1 to 2	180	100	585	89	83	26	7.6
	2 to 3	500	100	535	50	52	25	10.1
	3 to 4	500	100	735	58	53	30	14.7
	4 to 5	2620[3]	100	945	63	74	100	18.4

[1] Blood taken for analyses and weights of stools, if any, were not recorded, and hence are not included in calculations.
[2] Diluted seawater containing 257 m.eq. of Na, 295 m.eq. of Cl, and 5 m.eq. of K.
[3] Diluted seawater containing 176 m.eq. of Na, 202 m.eq. of Cl, and no K.

TABLE IIb

Balances of water, electrolytes, and nitrogen

Experiments IIA, IIB, and III

Expt.	Period	Intake								Output[12]					
		Oral								Urine					Blood
		H_2O	Carbohydrate	Whole fish and/or substitute						Vol.	Na	Cl	K	N	Vol.
				Wt.	H_2O	Na	Cl	K	N						
	days	*cc.*	*grams*	*grams*	*cc.*	*m. eq.*	*m. eq.*	*m. eq.*	*grams*	*cc.*	*m. eq.*	*m. eq.*	*m. eq.*	*grams*	*cc.*
IIA, Q	0 to 3 3 to 4	45[1] 2265[2]	 117							1430 530	261 2	255 13	157 39	27.3 9.6	75 90
IIB, Q	0 to 3	120[3]	320	720	990[4]	41	34	78	21.8	1480	240	129	80	26.8	85
IIA, C	0 to 3 3 to 4	50[1] 2070[2]	 82							1375 530	200 8	206 20	146 41	30.1 12.4	94 87
IIB, C	0 to 3	118[5]	25	565	862[4]	36	30	66	17.4	1770	279	295	238	31.5	85
IIA, H	0 to 3 3 to 4	0 1920[6]	 170							1715 460	305 3	243 20	157 27	31.0 10.6	78 109
IIB, H	0 to 3	130[3]	25	808	1062[4]	43	36	85	24.4	3000	536	475	141	37.7	85
IIA, T	0 to 3 3 to 4	0 1580[6]	 119							1378 460	165 2	176 7	118 34	25.0 10.8	65 97
IIB, T	0 to 3	93[7]	19		1015[8]	51	38	51	3.3	2370	334	341	149	28.3	85
IIA, R	0 to 3 3 to 4	0 985	 170							1645 540	284 11	269 21	97 20	24.7 7.0	65 109
IIB, R	0 to 3	45[9]	23		1500[10]	154	154			2540	392	396	112	24.4	85
III, H	0 to 2 2 to 4 4 to 6 6 to 7	800 0 160[11] 4300[6]	 25	 1584 1900	 1288 1555	 46 56	 41 50	 129 153	 45.0 54.0	1020 2115 2295 650	176 135 43 2	169 109 53 8	84 158 198 33	16.5 46.6 56.7 16.0	80 95 205 125
III, C	0 to 2 2 to 4 4 to 6 6 to 7	800 0 30 3270[6]								965 1030 995 450	62 79 29 3	60 64 47 9	64 84 95 32	18.0 27.5 24.4 11.4	80 100 185 125
III, T	0 to 2 2 to 4 4 to 5	800 0 2395								910 545 530	68 24 9	93 26 4	96 61 42	15.2 13.4 14.7	80 80 95
III, M	0 to 2 2 to 4 4 to 5	800 ? 2400		 805	 658	 23	 21	 65	 22.8	1810 1645 660	234 128 13	263 73 26	164 118 46	20.4 37.7 17.4	80 90 85

[1] Injected intravenously containing 1 per cent NaSCN.
[2] Includes 50 cc. of 1 per cent NaSCN solution injected intravenously.
[3] Includes 80 cc. of lemon juice containing 2.7 m.eq. of K, and the rest as 50 per cent glucose injected intravenously.
[4] Includes 400 cc. of "artificial fish juice" containing 20 m.eq. of Na, 15 m.eq. of Cl, 20 m.eq. of K, and 1.4 grams of N.
[5] Includes 68 cc. of lemon juice containing 2.7 m.eq. of K, and 50 cc. of 50 per cent glucose injected intravenously.
[6] Includes 50 cc. of 50 per cent glucose injected intravenously.
[7] Includes 55 cc. of lemon juice containing 1.9 m.eq. of K, and 38 cc. of 50 per cent glucose injected intravenously.
[8] Consists entirely of "artificial fish juice."
[9] 50 per cent glucose injected intravenously.
[10] Consists entirely of 0.6 per cent NaCl solution.
[11] Includes 60 cc. lemon juice.
[12] Subjects passed no stools, with exception of H, who passed 128 grams during the first period of experiment IIA, 90 grams during experiment IIB, and 6 grams during the first period of experiment III.

was started at 7 a.m., at which time the subject voided and was weighed, stripped of clothing. A sample of venous blood was then taken. All urine was collected with thymol and sulfuric acid to prevent the loss of ammonia. Small stools were passed by several subjects on the first day; these were weighed and discarded. The scales on which the subjects were weighed in Experiments II and III were accurate to 10 grams. Those used in Experiment I were less accurate and the 2 or 3 individual weights which were obviously grossly in error are so indicated in the tables.

The first group of experiments (Experiment I) was performed at Wright Field in March, 1943, the specimens of blood and urine being shipped to New Haven for all analyses except those for carbon dioxide. The subjects were allowed the activity of their usual military duties. Experiments II and III were carried out at New Haven in June and September, 1943, respectively. Activity was permitted as desired and decreased in most of the subjects after the first 2 days. Subject C was more active than his associates in both experiments. In Experiment II, sweating was induced in 2 of the subjects by controlled heat in a special constant temperature room for several hours at a time.

In Experiments I and IIB, carbohydrate was administered in the form of sugar. In Experiment IIB, the subjects ate raw fillet of haddock on the first and third days; on the second day, the raw fish was so repellant and nauseating that they were unable to take any. In Experiment III, during the first 4 days, cooked fish was substituted in order to insure the ingestion of a considerable amount. The closed dish containing the fish was weighed before and after steaming for 15 minutes. Occasional slight weight losses were corrected by the addition of water. One subject (M) developed abdominal distress and was unable to continue. The other subject (H) taking the same fish experienced no such reaction, although the cooked fish became increasingly distasteful. On the fourth day, raw fish was substituted and eaten with no greater relish.

TABLE III

Body weights, and analytical data for serum and blood

Expt.	Time from start	Body weight	Serum					Blood		Red cell
			Na	Cl	HCO_3	K	Total protein	NPN	Hematocrit	Protein
	days	*kgm.*	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*	*grams per cent*	*mgm. per cent*	*per cent cells*	*grams per cent cells*
I, L	0	85.0	138.1	101.1	20.1	4.04	6.79	29		
	2	81.4	138.0	99.1	18.9	3.45	7.32	42		
	5	78.2	130.5	98.3	18.5	4.17	7.53	42		
I, F	0	71.3	139.0	102.0	17.2	4.45	6.56	36		
	2	68.8	139.6	100.1	18.8	4.43	6.90	43		
	5	66.0	140.5	100.3	18.3	4.36	7.34	43		
I, Q	0	(73.6)†	134.0	97.5	20.1	4.64	7.01	32		
	2	(71.6)†	144.2	100.1	19.4	4.14	7.40	33		
	5	(71.5)†	130.2	100.3	19.3	4.21	7.61	31		
I, D	0	78.5	140.4	97.6	22.5	4.86	7.10	27		
	2	75.5	138.0	98.5	18.0	4.53	7.49	43		
	5	73.7	136.0	103.6	14.5	5.30	7.30	43*		
I, P	0	77.0	138.6	94.8	20.1	3.82	6.79	33		
	2	(76.1)†	142.8	99.7	19.5	3.52	7.48	40		
	5	72.7	136.2	97.3	19.8	4.36	7.41	44		
I, R	0	64.1	138.3	104.1	20.9	4.06	6.38	35		
	2	61.5	141.7	100.1	19.5	4.39	7.03	39		
	5	59.5	137.6	101.6	20.1	3.94	7.46	37		
I, W	0	70.0	138.0	96.9	19.5	3.66	6.63	32		
	2	67.3	134.7	99.2	18.5	4.65	7.28	34		
	5		138 1	98.3	20.5	4.37	7.10	36		
I, H	0	78.0	139.5	101.6	19.8	4.30	6.97	26		
	2	74.1	136.6	101.6	20.0	5.04	7.37	30		
	5	73.7	139.2	107.3	19.8	4.74	6.70	25		
IIA, Q	0	73.75	140.4	100.4	27.9	3.71	7.33	33	49.0	33.2
	3	68.25	143.3	99.8	24.1	5.36	8.09	34*	55.1	32.8
	4	69.10	137.4	95.2		3.62	8.17	33	52.1	31.3
IIB, Q	0	71.55	140.5	93.2	29.7	3.22	6.48	27	44.0	34.6
	3	67.83	145.9	103.3	31.4	3.56	7.98	40	47.1	34.7
IIA, C	0	72.16	142.0	100.4	26.8	3.16	6.38	40	56.2	33.3
	3	66.86	144.3	100.1	27.5	4.20	7.35	34	53.2	31.4
	4	67.65	142.7	95.7		3.50	7.38	28	51.5	31.8
IIB, C	0	71.47	141.8	103.5	26.7	3.87	6.52	31	45.4	35.1
	3	65.32	145.3	104.3	25.6	4.25	7.51	52	48.1	34.8
IIA, H	0	75.55	141.7	103.7	25.8	3.98	6.99	32	42.0	27.9
	3	71.00	144.8	101.4	23.4	5.14	7.74	40	50.0	27.3
	4	71.20	135.0	99.5		2.97	7.41	30	47.4	27.6
IIB, H	0	75.32	141.1	104.1	25.7	4.89	6.39	28	38.9	30.1
	3	69.51	141.5	100.5	24.8	4.65	7.84	41	47.5	30.0
IIA, T	0	76.98	143.6	98.2	29.1	3.67	7.32	28	51.5	34.0
	3	72.18	143.4	96.5	29.8	4.70	7.88	35	59.4	29.2
	4	72.29	137.7	94.9		3.90	7.63	33	52.3	33.5
IIB, T	0	76.08	137.7	101.2	27.8	3.86	6.68	27	47.4	33.6
	3	70.83	137.6	95.5	28.6	4.05	7.68	39	51.3	34.3
IIA, R	0	63.43	141.7	100.5	29.3	3.82	6.28	29	44.9	33.0
	3	59.59	139.1	97.1	25.2	3.95	6.93	38*	53.2	30.9
	4	59.31	138.0	96.8		3.90	7.05	28	52.2	31.3
IIB, R	0	62.94	140.8	104.1	27.4	4.15	6.22	26	42.5	33.6
	3	59.21	139.5	99.6	21.7	4.47	6.85	37*	45.3	34.4
III, H	0	75.26	144.1	101.5	26.2	4.91	7.12	28	39.0	26.0
	2	72.88	140.7	101.6	22.3	4.28	7.31	36*	41.1	28.6
	4	70.06	141.6	102.3	25.9	4.18	7.89	43	41.1	28.6
	6	68.14	151.1	104.7	28.1	4.10	8.42	53	41.9	27.7
	7	70.83	138.1	95.6	25.7	4.43	7.46	38	38.7	26.4
III, C	0	70.96	141.3	102.0	27.2	4.37	7.07	30	49.6	33.6
	2	68.86	141.6	98.0	25.9	4.36	7.31	41	55.2	32.6
	4	66.15	144.7	97.5	24.4	4.23	7.79	42*	53.8*	33.0
	6	63.53	147.4	101.6	25.5	4.55	7.70	45*	54.4*	31.4
	7	65.41	141.0	93.0	27.3	3.89	7.26	39*	51.2*	32.9
III, T	0	75.98	139.0	92.8	30.4	4.03	7.23	27	55.7	32.3
	2	73.29	142.6	95.6	30.2	4.34	7.49	32	57.1	32.3
	4	70.76	143.2	95.7	29.2	3.76	7.98	41	57.3	32.8
	5	71.73	137.7	91.2	26.8	4.40	7.76	39	56.7	32.1
III, M	0	75.36	141.0	103.6	26.6	4.22	7.11	30	51.4	32.7
	2	71.84	136.6	98.9	25.0	3.72	7.20	37	53.6	33.3
	4	70.91	140.8	95.9	25.5	4.03	7.51	37	56.4	33.2
	5	70.97	137.7	94.5	21.9	3.45	6.60	32*	52.0	33.1

* Blood ketone bodies greater than 15 mgm. per cent.

† The accuracy of these values is questionable.

TABLE IV

Insensible weight loss (IL) and changes in total body water (ΔW), in extracellular fluid (ΔE) and in intracellular fluid (ΔI_I and ΔI_{II})

Expt.	Period	IL	ΔW	ΔE	ΔI_I	ΔI_{II}
	days	*kgm.*	*liters*	*liters*	*liters*	*liters*
I, L	0 to 2	2.82	−2.85	−1.84	−1.01	−0.62
	2 to 5	3.27	−2.32	−0.33	−1.99	+1.30
I, F	0 to 2	2.42	−1.86	−0.70	−1.16	−0.88
	2 to 5	2.57	−2.11	−0.52	−1.59	−1.30
I, Q	0 to 2	(0.96)*	−1.74	−1.82	+0.08	−2.78
	2 to 5	(1.26)*	+0.24	−0.88	+1.12	+2.41
I, D	0 to 2	2.11	−2.44	−2.29	−0.15	−0.13
	2 to 5	3.07	−0.98	+0.91	−1.89	−0.72
I, P	0 to 2	(1.41)*	−0.64	−1.43	+0.79	−1.27
	2 to 5	(4.72)*	−2.32	+0.23	−2.55	+1.30
I, R	0 to 2	2.57	−2.03	−0.57	−1.46	−1.07
	2 to 5	3.23	−1.31	−0.31	−1.00	+0.31
I, W	0 to 2	2.91	−2.04	−1.80	−0.24	+0.17
	2 to 5			−0.46		
I, H	0 to 2	3.23	−3.15	−2.64	−0.51	−0.10
	2 to 5	2.04	−0.02	−0.46	+0.44	−1.15
IIA, Q	0 to 3	3.98	−4.78	−2.58	−2.20	−1.96
	3 to 4	1.66	+1.23	+0.40	+0.83	+1.09
IIB, Q	0 to 3	3.65	−2.65	−2.44	−0.21	−1.68
IIA, C	0 to 3	3.84	−4.58	−2.44	−2.14	−1.57
	3 to 4	1.44	+1.13	+0.30	+0.83	−0.04
IIB, C	0 to 3	5.33	−5.21	−2.99	−2.22	−2.08

Expt.	Period	IL	ΔW	ΔE	ΔI_I	ΔI_{II}
	days	*kgm.*	*liters*	*liters*	*liters*	*liters*
IIA, H	0 to 3	2.58	−3.73	−1.95	−1.78	−1.96
	3 to 4	1.46	+0.53	+0.07	+0.46	+2.18
IIB, H	0 to 3	3.88	−4.66	−3.47	−1.19	−0.69
IIA, T	0 to 3	3.32	−3.91	−1.45	−2.46	−0.87
	3 to 4	1.09	+0.33	+0.15	+0.18	−1.17
IIB, T	0 to 3	3.83	−4.21	−2.03	−2.18	−0.93
IIA, R	0 to 3	2.08	−3.28	−2.12	−1.16	−0.25
	3 to 4	0.49	−0.21	−0.17	−0.04	+0.25
IIB, R	0 to 3	2.57	−3.02	−1.71	−1.31	−0.93
III, H	0 to 2	2.04	−1.80	−1.55	−0.25	−0.03
	2 to 4	2.12	−2.50	−0.80	−1.70	−0.17
	4 to 6	1.38	−1.80	−0.48	−1.32	−2.40
	6 to 7	0.81	+2.96	+1.14	+1.82	+2.83
III, C	0 to 2	1.82	−1.57	−0.05	−1.52	−0.45
	2 to 4	1.54	−2.24	−0.62	−1.62	−1.35
	4 to 6	1.44	−2.13	−1.00	−1.13	−1.20
	6 to 7	0.79	+2.17	+1.07	+1.10	+1.12
III, T	0 to 2	2.47	−1.99	−1.36	−0.63	−1.52
	2 to 4	1.88	−1.98	−0.33	−1.65	−0.57
	4 to 5	0.78	+1.23	+0.60	+0.63	+0.90
III, M	0 to 2	2.37	−2.85	−1.72	−1.13	+0.12

* The accuracy of these values is questionable.

The experimental conditions imposed are summarized in Table I. Roman numerals refer to experiments, Experiment II being divided into 2 parts, A and B. The other letters refer to individual subjects.

RESULTS

Balance data are presented in Table II, the concentrations of various substances in serum and in blood in Table III. Table IV consists of the calculated total, extracellular, and intracellular water balances.

(*A*) *Changes in concentrations in serum and in whole blood*

Sodium of serum changed but little in the majority of the experiments. It increased 4 or more milliequivalents at some time during 7 out of 22 dehydration experiments, including the 2 in which the subjects perspired and the 2 in which water was withheld for 6 days. It decreased to a comparable extent in 2 others.

Chloride of serum increased 4 milliequivalents or more with dehydration in 4 instances and decreased to the same extent in 4 others.

Bicarbonate of serum was also usually little affected. In 3 cases, a slight reduction took place (I-D, IIA-R, IIB-R), associated with a marked rise of blood ketones. In Experiment III-C, on the other hand, bicarbonate was unaffected in spite of a comparable degree of ketonemia.

Potassium concentration in serum was usually virtually unchanged. It increased 1 milliequivalent or more only in Experiments I–W and in 4 of the 5 subjects of Experiment IIA.

Protein concentration in serum and *relative cell volume* in blood rose in all but 1 of the experiments in which dehydration occurred. This increase was not regularly progressive after the first 2 or 3 days, in spite of further dehydration.

Non-protein nitrogen concentration in blood

increased after 2 to 3 days of water restriction, with 1 exception. With further dehydration, the change was irregular. Ingestion of fish accentuated the rise.

(*B*) *Excretion of electrolytes and of nitrogen in urine*

Sodium and *chloride* continued to be excreted in considerable amounts during the first day or two after food and water were withdrawn. With each successive day thereafter, the excretion tended to diminish. Excretion of sodium tended to parallel that of chloride, but was not identical with it. No relationship was evident between urine volume and the excretion of these 2 electrolytes.

Potassium excretion remained at a high level throughout the experiments, although occasionally there was a slight decrease in the daily excretion of potassium after the initial period. On the whole, the ratio of potassium to nitrogen in the urine tended to exceed that found in skeletal muscle (10).

Nitrogen excretion continued at a high level throughout all experiments, and was correlated with urine volume (Figure 1). Three of the 4 subjects receiving carbohydrate in Experiment I had lower rates of protein metabolism than did the starving subjects, smaller amounts of nitrogen in the urine, and lower daily urine volumes.

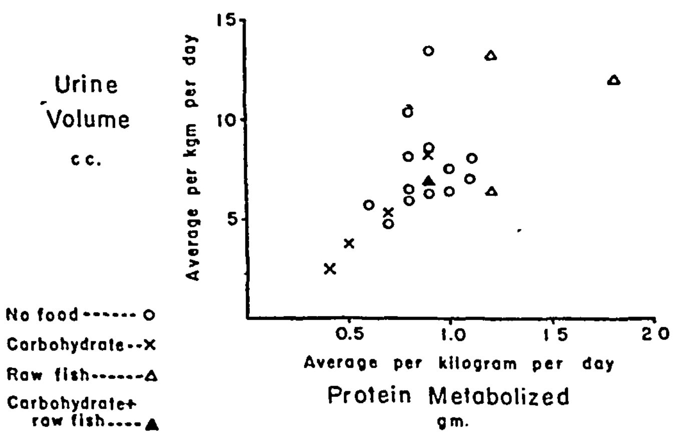

Fig. 1. Urine Volumes and Nitrogen Excretion in Subjects Partially or Completely Deprived of Water.

Average daily volume of urine is correlated with the average daily amount of protein metabolized as measured by the nitrogen excreted in the urine. Subjects receiving carbohydrate tended to conserve body protein and so to have low urine volumes.

The concentrations of nitrogen in the urine were, however, about the same as those in the starving subjects.

(*C*) *Metabolism of foodstuffs*

Rate of *protein* metabolism varied from subject to subject. Those taking carbohydrate usually, but not always, had a lower rate of protein destruction than did those receiving no food. Subject H (Experiment III–H) had an abnormally high rate of protein metabolism while receiving large amounts of fish, and was unique in that he was for a time almost in nitrogen equilibrium. Nitrogen balance was negative in all others.

Carbohydrate metabolism in starving subjects must have been reduced early to a low level, since ketosis usually developed within 2 days.

Fat metabolism continued at a high level, since most of the total caloric expenditure must have come from this source.

(*D*) *Water metabolism*

(*1*) *Total water exchanges*

A considerable deficit of water developed in all experiments. Limited ingestion of water during starvation ameliorated but did not abolish this loss. The ingestion of salt in hypotonic solution during the initial period of starvation in 1 subject (Experiments IIA-R and IIB-R) did not mitigate the water loss. Later ingestion of hypotonic saline when dehydration was more advanced did result in some retention of water (Experiments I-H and I-D). Carbohydrate ingestion decreased the rate of water loss in 3 of the 4 experiments in which adequate data are available (I-P, I-R, and IIA-Q), mainly because there was a lower daily loss of water in the urine (Figure 1). The subject eating large quantities of fish (III-H) lost water just as rapidly as did the control (III-C) who took nothing (Figure 2).

Water was lost through urine, skin, and lungs, fecal loss being negligible. It decreased slightly in Experiment III-C after 6 days of complete starvation. Unusually large losses through the skin developed only after deliberate induction of sensible sweating in subjects Q and C of Experiments IIA and IIB. Because of the stability of the daily IL in non-sweating subjects, total rate of water loss from the body was modi-

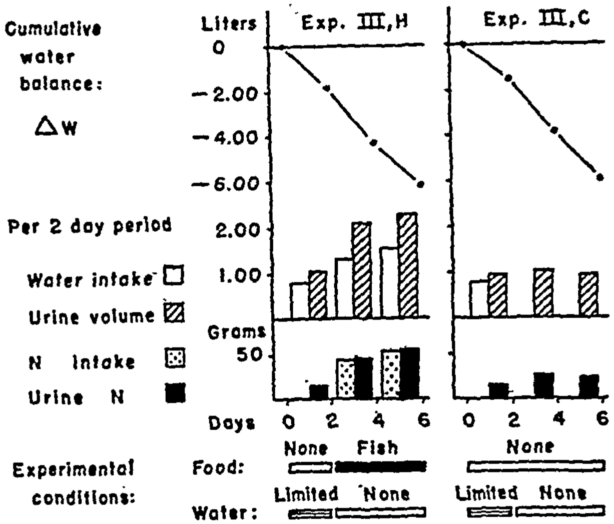

FIG. 2. INGESTION OF FISH BY A SUBJECT DEPRIVED OF WATER: FAILURE TO MITIGATE DEHYDRATION

Subject III-H ate fish while III-C served as a control. Water derived from the fish was not retained by subject III-H, so that both subjects lost water at approximately the same rate. Failure of water retention was apparently due to the greatly increased production of non-protein nitrogen, with consequent increase in the volume of urine used in its excretion.

fied only by some change in the relation of urinary volume to fluid intake, such as that following the ingestion of carbohydrate (Figure 1). If the ingestion of fluid was followed by an equal increase in the volume of the urine, as occurred after the ingestion of whole fish, no reduction in the rate of water loss resulted.

(2) Partition between extracellular and intracellular phases

Fluid was lost from both extracellular and intracellular phases. During the first 48 hours, extracellular loss usually predominated. This coincided with the period during which most sodium and chloride were lost in the urine. Later, the loss in terms of absolute amounts of fluid became predominantly intracellular, although in proportion to their respective initial magnitudes the losses from the 2 phases were about equal.

(3) Rehydration experiments

Experiments with rehydration in which water was offered *ad libitum* (final periods in all experiments of IIA and of III) indicate that the subjects retained only part of the water, even when considerable deficits of water were present. Almost always enough was retained to dilute the sodium of serum slightly below its initial concentration so that the body water became hypotonic. This was, of course, a consequence of the preceding salt loss. The 2 subjects who had perspired complained of abdominal cramps during rehydration. The water retained entered both phases.

(4) Water of erythrocytes

Using cell protein as an inverse measure of cell water (4), there is little correlation between water of erythrocytes (Table II) and change in total intracellular water (Table IV).

(E) Reactions of subjects

Thirst appeared early and regularly. After extended water deprivation, the thirst was quenched by the ingestion of an amount of water much less than that which had been lost. Lassitude was pronounced, and the subjects were often irritable and foolishly argumentative. These reactions were also present in the heavy fish eater (III-H), although starvation was much less and ketosis slight. The reaction to the ingestion of raw fish varied from distaste to nausea; no vomiting or diarrhea occurred.

DISCUSSION

Dehydration associated with starvation. Complete withdrawal of food and water results in dehydration through 2 mechanisms. In addition to the obvious negative water balance, there is a further loss of water associated with the starvation. Thus, in Benedict's experiment in which Levanzin lost in 3 days twice as much water as he ingested (13), and in some of our studies with dogs (12), fasting without water deprivation was accompanied by an increase in the negative water balance. This loss occurred through the maintenance of a large urine volume. It could not be cancelled by the ingestion of hypotonic saline (Experiment IIA-R). Sufficient electrolyte was excreted in the urine to prevent a rise, or to produce a decrease in the concentration of serum base. The concentrations of base and nitrogen in the urine were relatively low rather than maximal.

The mechanism of this initial diuresis is not clear. It does not appear to be the result of an increased production and excretion of ketone bodies. The diuresis appears quite early while ketosis is minimal. It then diminishes as ketosis increases. It is not the chief limiting factor in survival, since studies indicate that fasting animals allowed water *ad libitum* do not die of dehydration (14).

Effect of saline solutions in dehydration. Though it is known that concentrated salt solutions may provoke vomiting and diarrhea, the advisability of extending fresh water with sea water must be considered. Ladell (15) has shown that a small amount of the salt and water in a 62 per cent solution of sea water was retained during the first day by the dehydrated subject. Thereafter no further retention of salt was observed, and the negative water balance recurred. When the ingestion of the solution was discontinued, the salt and water retained initially were excreted. In our experiments with diluted sea water (I-H and I-D), a similar retention of water and salt occurred. Detailed analysis, however, of the combined dehydration and rehydration periods is not possible. Ladell believes that the reexpansion which occurs in the extracellular fluid volume is beneficial. Such solutions also permit, he states, the formation of the basal volume of urine with a decreased loss of body water. There is no proof, however, that any benefit accrues beyond that which could be obtained from the fresh water diluent alone. Moreover, the intake of diluted sea water is psychologically undesirable, since it would lead to the drinking of more concentrated solutions as the fresh water supply dwindled.

Effect of carbohydrate in dehydration. The anti-dehydrating effect of carbohydrate early in deprivation is in part the result of the water produced through its oxidation and in part the result of the protein-sparing effect. In Figure 1, the positive correlation between urine nitrogen and urine volume suggests that the amount of nitrogenous end-products is the chief limiting factor in the conservation of water through a decrease in the urine volume. Carbohydrate reduces the amount of nitrogen excreted.

The concentrations of blood ketones (9) indicate that carbohydrate ingestion is associated with an economy of body water before ketonemia becomes pronounced. Quantitative observations of urine ketones were not made. The decreased loss of water did not occur in the absence of the nitrogen-sparing effect, even though ketosis was inhibited (Experiment I-H). It is possible that with progressive ketosis, the excretion of ketone bodies assumes a greater rôle in the production of dehydration. During the early days of total deprivation, however, the nitrogenous end-products determine the volume of urine excreted.

Effect of ingested fish. Should fish be eaten by castaways with limited or non-existent water supplies? In Experiments IIIC and IIIH, subject H who ingested large amounts of fish without added water became dehydrated at exactly the same rate as the control (Figure 2). In these studies, only one species of fish was used and universal application of the results may not be valid. No difference could be detected between the effects of raw and cooked fish.

It would appear that fish eating did not ameliorate dehydration in man, since none of the water of the fish was retained, and the loss of the subject's own water was not curtailed. This can be attributed to the large volume of urine necessary for the excretion of the nitrogen of the fish protein. The sodium and chloride concentrations in the urine were very low and of no significance in increasing the urine volume. In the above experiments, the great similarity of the 2 curves of water loss may be fortuitous. The rate of dehydration might well vary with the species and composition of the fish. Furthermore, the ability of these 2 subjects to concentrate nitrogen in the urine might not be representative. These variables would increase or decrease the rate of water loss.

Since whole fish contained just enough water for the excretion of the metabolized nitrogen, the ingestion of fish by humans is definitely contraindicated under conditions of water deprivation. Dogs given dry protein became dehydrated much more rapidly than controls deprived of all intake (12). In contrast to humans, raw fish fillets without extra water maintained dogs in a vigorous state for at least 4 weeks (12). This difference between the human and dog subjects can be explained by the dog's greater ability to concentrate nitrogen, with conservation of some

of the water of the ingested fish. Unless the human subject can equal this ability to concentrate nitrogen, protein taken with insufficient water will be deleterious. Moreover, if the extrarenal water loss of the subject eating fish were increased by sweating, the same amount of water would still have to be sacrificed in the urine to excrete the nitrogen. Economy of water through the reduction of urine volume below that of the non-sweating subject is precluded. It seems likely, therefore, that men eating fish without additional water in tropical regions might on this account become more dehydrated than would those fasting without water.

With adequate water supplies to compensate for the dehydrating effect of protein, 2 clear advantages accrue to the fish eater. Ketosis is suppressed, and wastage of body protein is minimized.

SUMMARY AND CONCLUSIONS

1. Balances of water, nitrogen, and electrolytes have been studied during water deprivation, starvation, and the ingestion of various solutions and foodstuffs.

2. Fasting increased the negative water balance during water deprivation.

3. The loss of fluids and electrolytes occurred at first predominantly from the extracellular phase, and subsequently from the intracellular.

4. No clear advantage of hypotonic saline over fresh water could be demonstrated in the amelioration of dehydration.

5. Carbohydrate decreased the negative nitrogen balance, the ketone formation, the urine volume, and the dehydration of completely deprived subjects. Its water of oxidation was also made available to the body.

6. All of the water of ingested fish was used to excrete the protein metabolites and therefore failed to minimize dehydration.

7. Under conditions of limited water supply, ingestion of protein foods is definitely contraindicated. Carbohydrate is the foodstuff of choice.

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THE DELETERIOUS EFFECT IN DOGS OF A DRY PROTEIN RATION[1]

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The present study seeks to determine whether dry protein without additional water increases dehydration. It was undertaken in a search for information whereby the survival time of individuals with inadequate supplies of water could be prolonged. Dogs deprived of food only, or of all intake, served as controls. The experimental variants included dry protein, dry carbohydrate, mixtures of dry protein and carbohydrate, fresh fillets of fish, and 20 per cent urea solution. Balances of water, electrolytes, and nitrogen were determined and the physiological reactions of the animals observed.

MATERIAL AND METHODS

Adult dogs were used throughout. Fourteen animals were fasted without water as controls. Data from 8 of these experiments have already been published (1 to 3). Data from the other 6, including 4 rehydration periods, are included in Tables IA and IB. Five other animals were fasted but allowed water as desired. Eight animals received a thoroughly dried proprietary dog food, "SAS," in various amounts, enclosed in large gelatin capsules. One lot of dog food contained by analysis: 431 m.eq. of sodium, 275 m.eq. of chloride, 100 m.eq. of potassium, and 76.7 grams of nitrogen per kilogram. One kilogram of another lot contained the same amount of sodium and potassium, 292 m.eq. of chloride, and 73.8 grams of nitrogen. Both contained 9 per cent of fat, 10 per cent of bone, traces of carbohydrate, inorganic salts, and vitamin concentrates. This dog food constitutes under ordinary circumstances a complete maintenance ration. Empty capsules averaged 0.9 grams in weight and contained 152 grams of nitrogen per kilogram of capsule. Six to 18 capsules filled with dry ration and lubricated with mineral oil were swallowed after insertion into the hypopharynx. Vomiting was observed in only a few instances, contrary to the experience of Straub (4). Five dogs were fed carbohydrate and protein in the form of glucose enclosed in gelatin capsules without water. Three animals were given carbohydrate alone in the form of granulated sugar or of nitrogen free candy balls. Two dogs were fed fillets of raw haddock. By analysis of combined aliquots from 12 of the fish fillets, each kilogram contained: water 808 grams, sodium 29.8 m.eq., chloride 26.7 m.eq., potassium 87.3 m.eq., and nitrogen 28.4 grams.

All stool and urine specimens were analyzed for total nitrogen, potassium, sodium, and chloride. A small loss of ammonia from stools may have occurred during drying. Body weight, and the concentration of non-protein nitrogen in blood and of chloride and protein in serum were determined at intervals. In some experiments, analyses of serum for sodium and potassium were included. The

TABLE IA

Control experiments: fasting with and without water
Body weight, blood and serum analyses

Exp.*	Time	Body weight	Blood NPN	Serum: Total protein	Serum: Na	Serum: Cl	Serum: K
	day	*kgm.*	*mgm. per cent*	*grams per cent*	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*
23C	0	9.94	29	6.00	140.1	102.9	4.78
	7	8.12	35	6.98	146.5	109.0	4.63
30B	0	7.54	24	5.59	142.5	107.2	4.99
	10	6.00	31	6.69	151.6	113.0	5.38
32B	0	7.26	21	5.60	142.6	106.5	3.99
	7	6.12	40	6.81	146.3	110.6	5.37
35A	0	7.30	26	5.88	140.0	107.5	4.67
	10	5.86	29	6.53	149.3	114.4	5.07
35D	0	7.44	23	7.12	137.2	106.1	3.28
	15	5.36	28	6.29	153.3	121.3	4.69
	16	5.68	20	5.12	127.1	97.8	4.83
37C	0	7.56	26	7.12	141.5	105.4	3.53
	15	5.50	20	8.02	158.5	120.9	4.74
	16	5.78	18	6.51	127.7	97.5	4.85
23D	0	9.76	27	5.69	145.3	107.4	4.73
	8	8.26	27	6.07	146.1	110.7	5.00
32C	0	7.38	29	5.29	142.9	110.4	4.47
	8	6.22	18	5.65	145.2	107.4	5.30
30C	0	7.06	22	6.40	149.5	109.2	5.26
	14	5.52	30	5.60	146.9	108.0	5.74
35C	0	8.80	32	6.40	148.5	104.6	4.93
	15	6.48	31	5.55	141.0	109.7	4.38
37B	0	8.73	22	7.33	143.5	109.7	5.74
	15	6.62	30	6.33	138.5	104.4	5.08

* Water withheld or administered as indicated in Table IB.

[1] The authors are indebted to Dr. Samuel Harvey for the use of the Surgical Laboratory, and to Mrs. Theodore Mintz for technical assistance.

TABLE IB

Control experiments: fasting with and without water

Balances of electrolytes and nitrogen; calculation of insensible loss of weight (IL) and of changes in total water balance (ΔW), in extracellular fluid (ΔE), and in intracellular fluid (ΔI).

Exp.	Period from start	Water	Urine	Balance				IL	ΔW	ΔE	ΔI_I*
				Na	Cl	K	N				
	days	*cc.*	*cc.*	*m. eq.*	*m. eq.*	*m. eq.*	*grams*	*kgm.*	*liters*	*liters*	*liters*
23C	0 to 7	0	430	−56.6	−81.8	−75.4	−15.3	1.28	−1.35	−0.83	−0.68
30B	0 to 10	0	175	−39.2	−37.3	−77.6	−18.7	1.26	−1.10	−0.41	−0.69
32B	0 to 7	0	235	−28.0	−31.2	−44.8	− 8.3	0.89	−0.88	−0.31	−0.39
35A	0 to 10	0	205	−64.8	−33.8	−64.2	−14.1	1.15	−1.04	−0.33	−0.62
35D	0 to 15	0	520	−31.4	−46.3	−68.2	−22.6	1.28	−1.47	−0.55	−0.73
	15 to 16	1450	715	− 8.9	−12.7	−22.2	− 2.7	0.35	+0.42	+0.21	+0.29
37C	0 to 15	0	553	−62.7	−37.5	−75.0	−23.6	1.28	−1.41	−0.52	−0.80
	15 to 16	730	170	−11.2	−14.2	− 9.4	− 3.0	0.23	+0.36	+0.23	+0.44
23F	0 to 3	0	150		−22.0	−25.0	− 6.2	0.58	−0.59	−0.28	−0.14
	3 to 4	590	280		− 5.0	− 5.9	− 3.0	0.29	−0.08	+0.14	+0.05
32E	0 to 3	0	138		− 7.0	−21.2	− 4.9	0.28	−0.34	−0.09	−0.21
	3 to 4	310	220		− 4.7	− 2.0	− 2.6	0.08	−0.07	0.00	+0.11
23D	0 to 8	1125	720		−51.2	−73.1	−16.6	1.81	−1.06	−0.49	−0.50
32C	0 to 8	2330	2050		−29.6	−55.4	−12.6	1.18	−0.74	−0.21	−0.42
30C	0 to 14	815	595	−44.0	−42.3	−69.4	−23.6	1.70	−0.99	−0.32	−0.42
35C	0 to 15	1870	1480	−63.0	−50.1	−92.8	−23.9	2.30	−1.60	−0.49	−0.41
37B	0 to 15	1500	1210	−67.5	−63.1	−94.8	−29.1	2.17	−1.51	−0.32	−0.42

* ΔI_I omitted, since it is simply $\Delta W - \Delta E$.

water content of serum was calculated from the total protein concentration by the formula: $W_s = 99.30 - 0.889\ P$. This formula was derived from simultaneous determinations of total protein concentration and water content of 54 samples of dog serum in previous experiments. Changes in total body water, in extracellular fluid volume, and intracellular fluid volume were calculated by methods previously described (1). Extracellular fluid changes were based on chloride balances.

RESULTS

Data on control animals deprived of food and, in some instances, of water as well, are presented in Tables IA and IB. Data from the animals which received various foodstuffs are recorded in Tables IIA and IIB. In Figures 1 and 2, the amount of protein metabolized daily is correlated with the survival time and with the daily water loss through various channels.

(*A*) *Period of survival.* There is a rough inverse proportion between the amount of protein metabolized daily and the period of survival. All but 2 of the control animals were resuscitated after 10 to 20 days of complete water deprivation. Survival was shortest in the animals with the highest daily metabolism of protein. The protein metabolized was not directly proportional to the protein ingested, but did increase with it.

(*B*) *Rate of water loss.* (Figure 2.) The average daily rate of water loss was much more rapid in the protein-fed dogs than in the controls. If the 2 dogs which received the smallest daily allotment of protein are excluded, the mean daily balance of water in the 6 other dogs was -29.1 ± 4.3 cc. per kilogram per day. The difference between this and the control value of -16.6 ± 3.2 is statistically highly significant. This increased rate of total water loss in the protein-fed dogs resulted from a larger daily urine volume. An increase in the daily insensible loss of water as a result of increased metabolism

may have been a contributory factor. The daily urine volume was practically doubled in the protein-fed dogs. This was associated with a higher daily metabolism of protein necessitating a greater excretion of nitrogen. It is evident that dry protein does spare body protein but it accelerates dehydration.

Animals fed carbohydrate alone, or together with the small quantity of protein of the gelatin capsules, resembled, on the whole, the controls in the amount of nitrogen metabolized, the volume of urine, and the total loss of water. In Dog 23, represented in Figure 1 by the cross farthest to the left, the carbohydrate definitely spared body protein, decreased urine nitrogen, and diminished dehydration. Experiments with this same animal deprived of all intake, deprived of food alone (23C and 23D, Tables IA and IB),

TABLE IIA

Ingestion of various foodstuffs without supplementary water

Body weight, blood and serum analyses

Exp.*	Period	Body weight	Blood NPN	Serum			
				Total protein	Na	Cl	K
	day	*kgm.*	*mgm. per cent*	*grams per cent*	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per liter*
12D	0 14	12.20 9.31	25 105	6.65 7.55	144.5 188.8	110.9 147.2	3.33 5.40
13D	0 8 13	11.20 9.08 8.11	29 28 130	6.29 7.67 9.65	141.9 162.7 190.3	109.3 129.3 158.3	4.49 5.32 6.08
23G	0 3 10 13	11.46 10.06 8.41 9.52	35 35 51 24	6.62 7.58 8.38 5.37	144.9 152.2 188.5 144.0	105.1 109.1 146.6 110.0	5.37 4.52 4.18 4.36
32F	0 3 5	7.38 6.52 5.89	31 48 98	6.49 7.76	149.8 172.1	113.1 133.9	4.94 4.47
39	0 7	11.08 8.76	30 98	6.60 8.07		104.8 160.6	
38	0 7	11.22 8.36	31 63	6.69 6.93		101.9 145.3	
41	0 9	6.90 5.12	32 38	5.80 6.96		109.9 144.1	
40	0 7	6.42 5.17	27 99	7.13 8.89		104.0 149.8	
23E	0 6	6.63 5.00	28 37	6.58 7.63	146.1 150.8	99.3 107.8	4.47 5.10
32D	0 6	4.37 3.80	23 39	6.76 6.50	146.2 148.3	105.5 106.5	4.73 5.15

TABLE IIA—*Continued*

Exp.*	Period	Body weight	Blood NPN	Serum			
				Total protein	Na	Cl	K
	day	*kgm.*	*mgm. per cent*	*grams per cent*	*m. eq. per liter*	*m. eq. per liter*	*m. eq. per l*
23H	0 11 28	10.10 9.70 9.22	24 26 28	6.35 6.66 6.44	143.6 147.8 144.5	105.4 108.3 110.5	4.1 5.1 5.1
37A	0 12 28	7.92 7.16 6.72	23 25 28	5.73 6.72 7.12	146.5 145.0 148.0	105.7 108.4 103.9	4.3 4.0 4.9
30A	0 8	7.62 6.34	33 32	5.84 6.48	149.4 147.5	106.3 111.1	4.6 5.0
31	0 7	6.76 5.51	27 25	5.06 7.17	145.9 167.0	108.8 126.6	4.6 5.9
35B	0 8	6.96 5.74	31 32	6.41 6.21	147.5 150.7	102.6 114.6	5.2 4.4
23L	0 9	11.45 9.37	30 22	6.04 7.26	149.5 160.0	105.2 113.9	5.2 3.9
37G	0 9	8.90 6.88	30 24	6.05 8.36	144.9 160.3	109.3 118.4	5.0 5.2
23M	0 9	10.11 8.86	22 22	6.08 6.56	145.4 151.6	105.3 116.9	
47A	0 6	6.76 5.79	24 31	4.96 4.95	142.8 153.4	105.8 118.0	
49A	0 9	16.29 13.80	29 38	6.09 7.34	148.8 160.3	113.7 115.2	

* Ingestion of dry protein alone in experiments 12 through 40; of urea in saline in 23E; of urea in 32D; raw fish in 23H and 37A; of protein and carbohydrate 30A through 37G; and of carbohydrate alone in 23 through 49A.

given protein alone, and given carbohydrate wit protein (23G and 23L, Tables IIA and IIB indicate clearly that an economy of body wate resulted from the administration of carbohydrat It is probable that carbohydrate produced similar effect in the other animals, but that th change was not large enough to place the animal outside the range of the control groups. It wa not possible to represent this graphically, sinc these points overlay those of the control group

(*C*) *Renal response.* In general, the urine vol umes and the concentrations of nitrogen in th urine were greater in the dogs fed protein tha in the controls. Experiment 32D is the sol exception. This dog had the largest urine vol ume and the shortest survival, despite a ver low concentration of nitrogen in the urine. It i

TABLE IIB

Ingestion of various foodstuffs without supplementary water

Balances of electrolytes and nitrogen; calculation of insensible loss of weight (IL) and of changes in total water balance (ΔW), in extracellular fluid (ΔE), and in intracellular fluid (ΔI).

Expt.	Period	Intake	Nitrogen			Urine vol.	Balance			IL	ΔW	ΔE	ΔI_{II}**
			Ingested	Excreted in urine	Excreted in stool		Na	Cl	K				
	days	*grams*	*grams*	*grams*	*grams*	*cc.*	*m. eq.*	*m. eq.*	*m. eq.*	*kgm.*	*liters*	*liters*	*liters*
12D	0 to 14	Dry protein	36.6	37.0	5.1	435	+77.8	+ 26.1	− 6.06	2.59	−2.36	−0.61	−1.46
13D	0 to 8 8 to 13	Dry protein Dry protein	20.8 13.0	30.7 15.9	1.9 2.3	680 265	−34.3 +50.2	− 38.3 + 25.3	−80.0 −17.0	1.58 0.71	−1.84 −0.80	−0.72 −0.28	−1.07 −0.66
23G	0 to 3 3 to 10 10 to 13	Dry protein Dry protein Dry protein	16.4 37.1 15.9	19.0 45.2 17.4	 8.0 3.5	405 670 440	−51.7 +61.0 +21.1	− 69.5 + 4.5 + 10.1	−35.7 −58.4 + 1.6	0.99 1.06 0.71	−1.15 −1.50 +1.32	−0.69 −0.54 +0.68	−0.43 −1.11 +0.96
32F	0 to 3 3 to 5	Dry protein Dry protein	16.1 8.8	15.1 3.6	 3.7	455 120	− 9.8	− 20.3	−37.8	0.46 0.46	−0.81 −0.48	−0.43	−0.57
39	0 to 7	Dry protein	62.3	44.2	6.5	925		+ 5.0		1.55	−2.13	−0.95	
38	0 to 7	Dry protein	75.7	59.3	9.8	1135		− 65.0		1.86	−2.56	−1.24	
41	0 to 9	Dry protein	59.6	38.3	9.6	620		+ 17.0		1.31	−1.62	−0.32	
40	0 to 7	Dry protein	51.5	35.2	6.6	550		+ 29.0		0.97	−1.28	−0.34	
23E	0 to 6	20 per cent urea in 0.9 per cent NaCl	13.8	25.4		740		− 64.7	−82.4	1.38	−1.63	−0.76	−0.83
32D	0 to 6	0.9 per cent NaCl	0	8.6		280		− 15.6	−44.4	0.62	−0.57	−0.14	−0.34
23H	0 to 11 11 to 28	Raw fish Raw fish	133.7 206.6	127.4 188.0		2450 3100	+ 3.0 −14.4	+ 4.7 + 33.0	+28.8 +42.0	2.29 4.11	−0.56 −0.61	−0.04 +0.21	+0.06 +0.19
37A	0 to 12 12 to 28	Raw fish Raw fish	59.3 97.2	45.6 78.1		1100 1650	−12.0 + 6.1	− 10.9 + 16.6	+10.8 +61.1	1.43 1.89	−0.58 −0.50	−0.16 +0.12	+0.10 +0.33
30A	0 to 8	Carbohydrate 193*	6.3	18.7	1.4	285		− 37.8		1.16	−1.05	−0.38	
31	0 to 7	Carbohydrate 171*	5.5	11.2	3.1	225		− 22.3		1.19	−1.02	−0.41	
35B	0 to 8	Carbohydrate 183*	5.5	13.2		390		− 37.2		1.11	−1.06	−0.46	
23L	0 to 9	Carbohydrate 564*	17.7	28.0		440		− 77.0		2.20	−1.82	−0.78	
37G	0 to 9	Carbohydrate 565*	16.6	28.4		1025		−105.9		1.49	−1.93	−1.00	
23M	0 to 9	Carbohydrate 562*	0	11.0		310		−110.1		1.27	−1.05	−1.10	
47A	0 to 6	Carbohydrate 360*	0	11.1		305		− 25.9		0.96	−0.89	−0.37	
49A	0 to 9	Carbohydrate 539*	0	28.8		685		− 85.2		2.20	−2.11	−0.67	

* Received dry carbohydrate in amounts noted plus protein as gelatin capsules.
** ΔI_I omitted, since it is simply $\Delta W - \Delta E$.

probable that this animal had latent renal insufficiency, not evident prior to the experiment. These studies indicate that the high nitrogen concentrations found in urine during water deprivation are not maximal, and that they can be increased by the administration of protein. However, this ability to increase nitrogen excretion without further water loss is limited, since the urine volume invariably increased with a higher daily metabolism of protein.

The concentration of non-protein nitrogen in the blood is an indirect measure of renal activity.

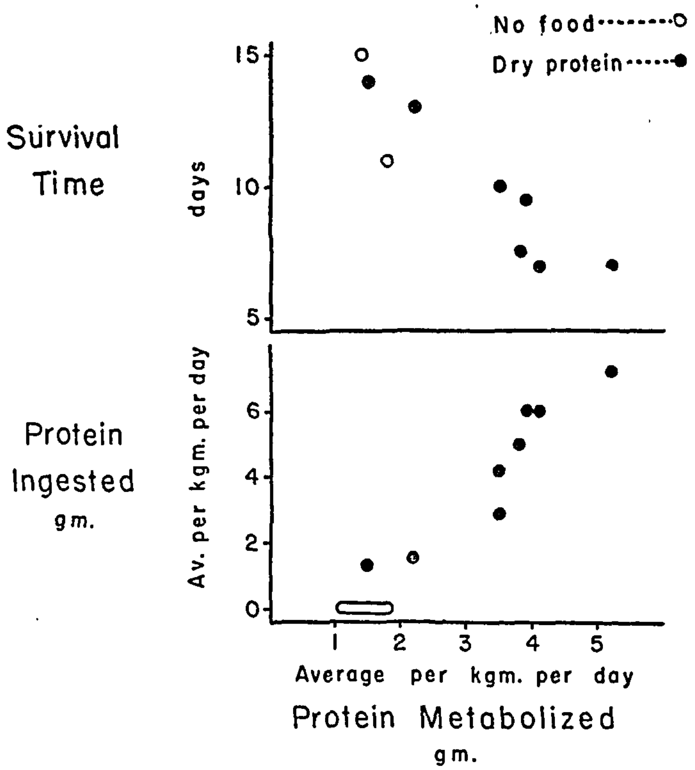

FIG. 1. PROTEIN METABOLIZED IS CHARTED AGAINST PROTEIN INGESTED AND AGAINST SURVIVAL TIME

As the metabolism of protein increased, the survival time decreased. After a survival period of 10 to 20 days, all but 2 of the control animals were resuscitated and hence do not appear in the upper graph. In the tables, periods of only 7 to 12 days are used for these animals for comparison with the feeding experiments.

In Figure 3, all values of blood non-protein nitrogen in the protein-fed dogs are plotted against time. The response was variable, since the non-protein nitrogen rose early in some and late in others. Apparently, in the majority, renal function was well maintained in spite of severe dehydration.

(*D*) *Distribution of water loss.* Control animals, as well as those maintained on dry protein, dry carbohydrate, or both, lost water from both phases. In percentage of initial volume, extracellular loss preponderated in about half of the animals; the intracellular loss was greater in the other half.

There is only a partial correlation between decline in the volume of extracellular fluid and an elevation in blood non-protein nitrogen. The extracellular fluid volume decreased early, and preponderated, in 3 of the 4 experiments with an early rise in blood non-protein nitrogen. In 3 of the 4 experiments with a late increase in the non-protein nitrogen, the loss from the intracellular phase was larger. These results suggest that a decrease in extracellular fluid volume affects renal circulation adversely.

The water loss in the dogs which received urea was distributed over both phases. The animals which were fed fish appear to have lost only intracellular water. There is no ready explanation for this unusual finding.

(*E*) *Clinical course.* Although the control animals lived longer than did the animals fed protein, the clinical course was similar. An accelerated weight loss was the only difference detected in the animals on protein ration. Vomiting and diarrhea were absent. The protein-fed animals continued to have formed stools, while the controls did not. The animals of both series tended to shiver, became very weak, and finally unresponsive.

DISCUSSION

Animals deprived of food and water rapidly become dehydrated, losing 27 to 36 per cent of their initial body water in 2 to 3 weeks (Table IB). Dehydration develops even in starving animals permitted free access to water, but it is not as severe (Table IB). Protein fed in excess of 1.5 grams per kilogram per day stimulates protein metabolism, accelerates water loss, and shortens the period of survival (Figure 1). Ingestion of smaller amounts of protein fails to increase protein metabolism (Figure 1), and hence does not accelerate water loss. The deleterious action of protein ingestion on water balance can be related to an increased urine volume consequent upon an increased formation of urea. The demand for the excretion of the additional urea is so imperative that water is sacrificed, even by the severely dehydrated organism. The organism does not conserve water by retention of urea, even though retention of water is a critical necessity. The increase observed in blood non-protein nitrogen was never sufficient to account for more than a fraction of the nitrogen metabolized.

Increase in the insensible loss of weight may sometimes contribute to the accelerated water loss following protein ingestion. It is reasonable to ascribe this to an increase in total metabolism, secondary to the increased protein metabolism.

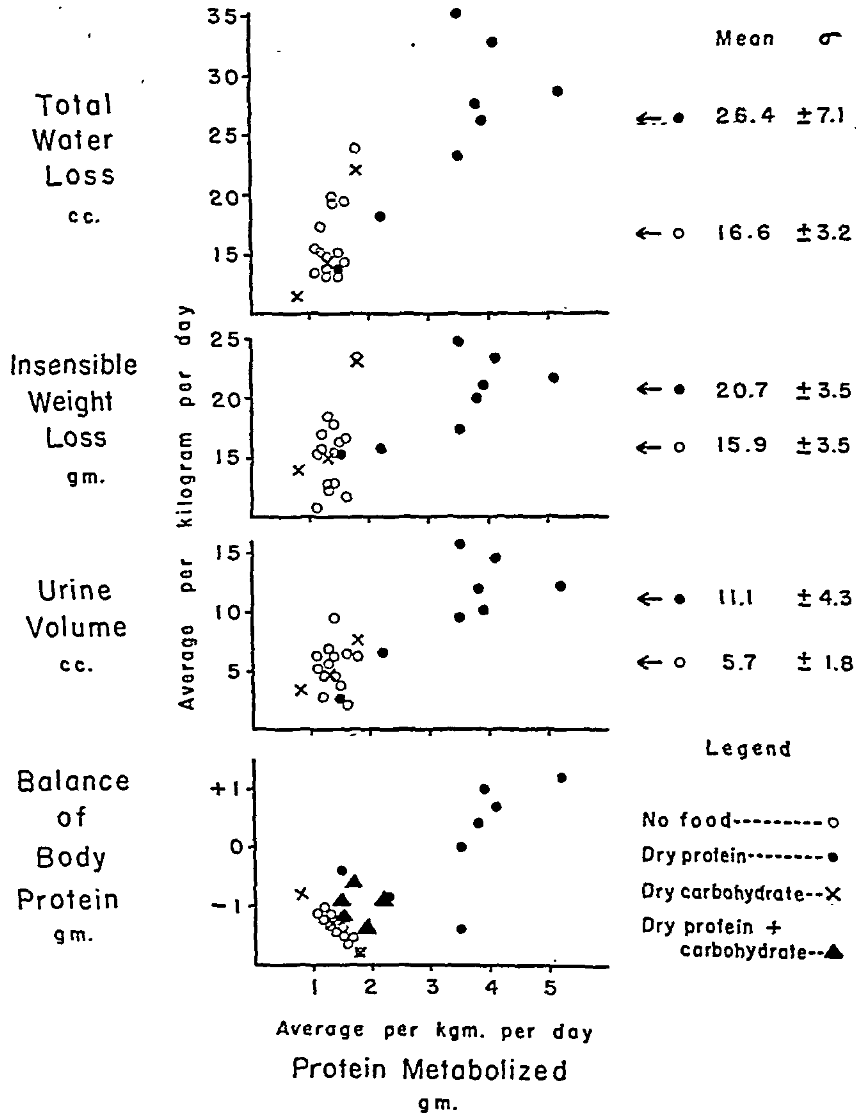

FIG. 2. PROTEIN METABOLIZED IS CHARTED AGAINST TOTAL WATER LOSS, INSENSIBLE WEIGHT LOSS, URINE VOLUME, AND BALANCE OF BODY PROTEIN, EXPRESSED AS AVERAGES PER KILOGRAM PER DAY

The animals fed dry protein plus carbohydrate are only plotted in the lowest figure since they overlie the control groups. It is evident that dry protein may spare body protein but accelerates dehydration. In dogs, carbohydrate ingestion diminishes dehydration chiefly by sparing body protein.

There is evidence, however, that the state of hydration plays a significant rôle as well in determining the amount of weight lost insensibly. Thus, the insensible loss of weight in dogs fasted without water was, on the average, 20 per cent lower than in dogs fasted with water. This difference is correlated with a greater water loss in the animals deprived of food and water. It seems quite as likely that this difference results from an alteration in the usual relationship between insensible loss and total metabolism with severe dehydration, as that total metabolism itself is altered in dehydration.

Since insensible loss of weight is used in calculation of total calories and in turn in the estimation of fat burned and of water lost, an alteration

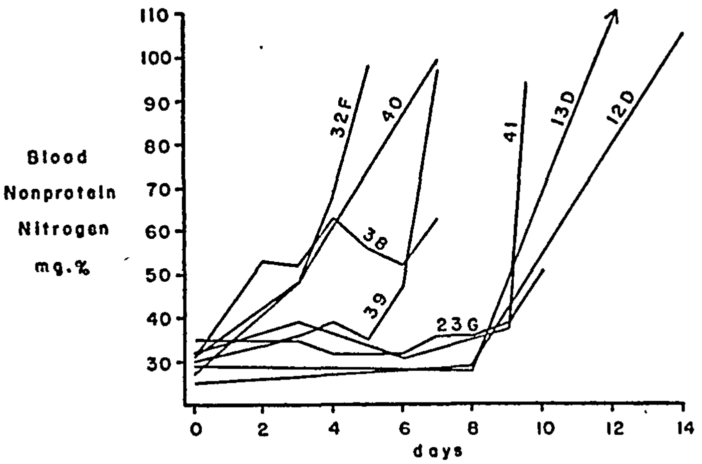

FIG. 3. BLOOD NON-PROTEIN NITROGEN CONCENTRATIONS IN THE DOGS FED DRY PROTEIN

Each line is based upon periods in individual experiments. The numbers of the experiments are identical with those in Table II. In the table, however, some of the intermediate periods have been omitted for the sake of economy.

in the usual relationship between total metabolism and insensible loss would affect the calculation of water balance. The alternative method for calculation of total calories independent of insensible loss, based on the calorimetric experiments of Anderson and Lusk (5), did result in higher values. In terms of water balance, the difference was too small to be significant.

These experiments clearly indicate that the water formed from the metabolism of protein together with that formed from the small amount of fat in the ration is insufficient to supply the extra water needed for the renal excretion of nitrogenous end-products. They also indicate that the dog cannot excrete the large amounts of extra urea without increasing the urine volume. Hence, the ingestion of protein food is advisable only if adequate amounts of water are available. As soon as water was administered at the close of Experiment 23G, the moribund animal recovered promptly, although the ingestion of protein was continued. Dogs remained in excellent condition, save for a slight weight loss, on a high protein intake of raw fish with no other source of water. Dogs placed on a similar intake of dry protein died even more rapidly than did those without any food or water. Therefore, only amounts of dry protein which do not increase protein metabolism above that of the fasting animal can be tolerated. In dogs, carbohydrate ingestion can benefit water balance by making available water of oxidation, and especially by sparing body protein. This reduces the urine volume by decreasing the amount of nitrogen requiring excretion. It is not possible to answer the general query as to whether carbohydrate can conserve body water by amelioration of ketosis, since marked ketosis does not develop in these animals (6).

These results with protein administration to dogs without water are applicable to human subjects (7). Since man cannot concentrate nitrogen to as great a degree as the dog (8), an even greater loss of water following the ingestion of dry protein might be expected.

SUMMARY AND CONCLUSIONS

(*1*) Dry protein in small amounts (1.5 grams per kilogram) without water maintains dogs in nitrogen equilibrium without change in the nitrogen excretion.

(*2*) Larger amounts of protein (2.0 to 7.2 grams per kilogram) increase the metabolism of protein and the nitrogenous end-products in the urine.

(*3*) The increased excretion of nitrogen necessitates a larger urine volume, even when dehydration is present.

(*4*) Dry protein in amounts greater than that necessary for nitrogen equilibrium, without adequate supplies of water, increases dehydration and decreases survival time.

(*5*) Similar amounts of protein with an adequate intake of water (whole fish) will maintain dogs in an excellent condition for at least 4 weeks.

(*6*) In the dog, administration of carbohydrate can conserve water. The economy of body water results chiefly from a decrease in protein metabolism and in the amount of nitrogen necessitating excretion. In addition, the water of oxidation of the carbohydrate is made available.

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BLOOD KETONES AND SERUM LIPIDS IN STARVATION AND WATER DEPRIVATION [1,2,3]

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Few observations of the behavior of serum lipids in acute human starvation have been reported and no studies using present improved analytical methods are available. The occasional published reports of changes in serum lipids in fasting experimental animals are often contradictory (1 to 9). Increases in the blood ketone bodies during starvation are well recognized (10 to 15), but have never been systematically related to serum lipid changes.

In this study, serum lipids and blood ketones were determined simultaneously in 14 fasting normal male human subjects, some of whom were also deprived of water. These have been supplemented with experiments on fasting monkeys and dogs. Some effects of feeding carbohydrate or fish to otherwise fasting subjects have also been included.

EXPERIMENTAL PROCEDURE

Fourteen normal adult male subjects were starved for periods of 2 to 6 days. Three of them, Yale medical students, were subjected only to a 2-day fast and were allowed water or black coffee *ad libitum.* The other 11 volunteers, members of the Army of the United States, were subjected to water restriction or deprivation. Some of them were subjects of carbohydrate and of fish feeding experiments as well. The organization of these experiments has been described in detail in an accompanying paper (16), and is summarized in Table I of that paper. Serum proteins were not determined in the medical students and serum lipids were not determined in the subjects eating fish. Intravenous glucose tolerance tests (17) with simultaneous blood ketone determinations were made at the end of 8 starvation periods in Experiments II and III and blood ketones were repeated in 3 of them after 24 hours of carbohydrate ingestion. After 2 periods of fasting, blood ketones were determined before and after 24 hours of sugar eating. Intravenous glucose tolerance tests were also carried out at the end of 4 experiments with fish feeding.

Four male *rhesus* monkeys (*macaca mulatta*) and 7 dogs were studied. The monkeys were starved for 3 to 6 days, the dogs for longer periods. All animals, with the exception of 2 dogs, were permitted to drink water *ad libitum.* In other experiments, the monkeys were allowed to eat varying amounts of sugar for 3 to 8 days.

CHEMICAL METHODS

Serum separated from clotted venous blood drawn under oil was used for all lipid analyses, while oxalated whole blood was used for the ketone determinations. In Experiment I, most of the analyses were carried out on blood or serum which had been transported for about 24 hours in special cooled containers. This procedure was justified by control experiments in which determinations of serum lipids and blood ketones in blood samples precipitated immediately were compared with those in blood samples precipitated after 48 hours of refrigeration. No significant change was found. The extra manipulations and transportation prevented duplicate analyses in a few instances and may have been responsible for some inconsistencies in the data of Experiment I.

[1] Lt. Col. D. B. Dill, Q. M. C., A. U. S., was responsible for the inception of this work and guided it through its initial stages. Capt. J. M. Quashnock, M. C., A. U. S., assisted in the management of the experiments and in the analysis of the data, as well as serving as a volunteer subject. Capt. Wilson, A. C., A. U. S., Lt. Holmes, Sn. C., A. U. S., Lt. Chovnick, A. C., A. U. S., Sgts. Tressler and Roberts, and Pvts. Perzanowski, Leyzorek, and McHugh, all of the A. C., A. U. S., served as volunteer subjects. We are indebted to Dr. T. S. Danowski, Dr. A. J. Eisenman, Dr. J. R. Elkinton, and Dr. E. A. H. Sims of the Department of Internal Medicine of Yale University School of Medicine, for their cooperation and help. Sugar and protein analyses were done by members of the laboratory staffs at Wright Field and at Yale. Miss Barbara Russell of the Yale University School of Nursing and Dr. S. C. Harvey of the Department of Surgery provided proper facilities for the care of the subjects. The work was carried out in connection with an Army Air Corps Contract.

The authors are indebted to Professor John Fulton who generously placed the facilities of the Laboratory of Physiology of the Yale University School of Medicine at their disposal.

[2] This article has been cleared for publication by the War Department Bureau of Public Relations. The opinions expressed are those of the authors and do not necessarily reflect official views of the War Department.

[3] The ketone investigation was aided by a grant from the Dazian Foundation for Medical Research.

Serum lipids, proteins, and blood sugars were determined by methods previously described (17 to 23). Neutral fat concentration was calculated by the formula of Peters and Man (24). The values for neutral fat calculated in this way are less accurate than are the values for the other lipid fractions, since the calculation introduces cumulative errors from 3 chemical methods and from variations in composition of phospholipids. Nevertheless, in each of 3 normal males, the greatest individual deviation from the average neutral fat of that individual was only ± 0.8 m.eq. per liter (24, 25). Whole blood ketones were determined by the method of Weichselbaum and Somogyi (26), with a combination of the procedures used in deproteinization and desaccharification. Results are expressed as mgm. per cent of acetone. At concentrations below 1 mgm. per cent, the proportion of known amounts of acetone recovered from blood was considerably less than that reported by the original authors. This is not a serious practical difficulty, however, since in this range a large percentage error represents only a small absolute one. At higher concentrations, the proportion recovered was similar to that reported by Weichselbaum and Somogyi (26). Nitrogen, water, and electrolyte balances were also measured in the military subjects and are reported elsewhere (16).

RESULTS

A. Normal human subjects

The data from all fasting normal subjects are contained in Table I. Subject T was atypical throughout, in that his carbohydrate tolerance, urinary nitrogen excretion, blood ketones, and serum lipids were little affected by starvation. Subjects D and H drank large amounts of dilute salt water on the fifth day of Experiment I, thereby certainly affecting the concentration of serum lipids at the close of the period. Data from all experiments with T and from these experiments with D and H are therefore considered separately, and the general statements made below concerning the results do not apply to them. In analyzing the data concerning lipids and proteins of serum, each change is referred to the initial concentration in the blood of the individual in the experiment under consideration.

Total cholesterol increased progressively in starvation. At the end of 2 days, the average rise did not usually exceed the average variation observed in normal subjects, ±13 mgm. per cent (derivation of normal average variations of cholesterol and lipid phosphorus is given under Figure 1). After 3 to 6 days, however, it amounted to 36 ± 13 mgm. per cent, a highly significant increment. This is illustrated in

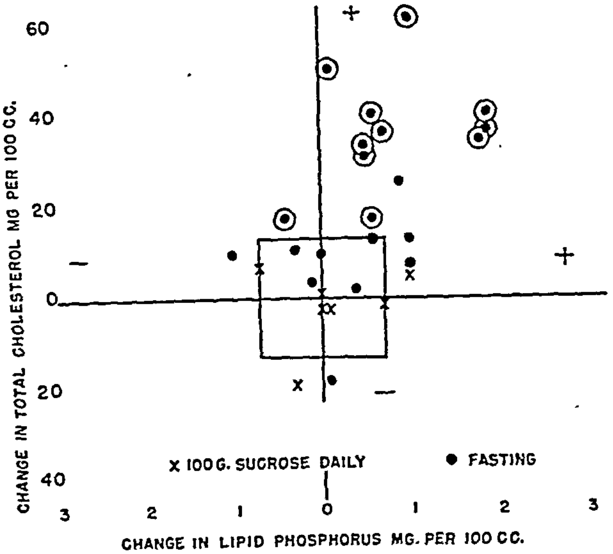


FIG. 1. THE RELATIONSHIP OF CHANGES IN SERUM TOTAL CHOLESTEROL TO THE CHANGES IN LIPID PHOSPHORUS

Dots represent the changes in fasting human subjects, crosses the changes in humans who ingested 100 grams of sugar daily. Circled dots denote values after 3 or more days of starvation. The square in the center denotes the average individual variation for the respective lipid fractions.

The probable error of the averages of duplicate total cholesterol determinations is 5.42 mgm. per cent (25). However, the variation in the individual from day to day is probably larger and would include the error of the method. In our own experimental work on 3 healthy male adults, in each subject, the average deviation from his average value did not exceed ± 13 mgm. per cent of serum total cholesterol (24, 25). Sperry (27), in 25 normal adults, found the variation of the individual from the average not to exceed ± 12.3 mgm. per cent, a value in close agreement with our value of ± 13 mgm. per cent.

In the same 3 normal males, the greatest average deviation from the average lipid phosphorus of any one individual was ± 0.7 mgm. per cent (24, 25). This includes the error of the method of ± 0.13 mgm. per cent (25).

Figure 1 in which the 11 observations after 3 or more days of starvation are distinguished by circled dots. In addition, it will be noted in this figure that cholesterol increased to some extent in all but 1 of 21 determinations, irrespective of the duration of starvation. The increase affected both free and esterified fractions of cholesterol. Although in the experiments of 3 or more days, the average increment of esterified cholesterol, 24 ± 11 mgm. per cent, is the greater, the average increase of free choesterol, 13 ± 3.6

TABLE I

Serum lipids and blood ketones of human subjects

Subject	Days	Serum lipids					Ketones as acetone	Total proteins	Food***	Fluid****
		Cholesterol		Lipid phosphorus	Fatty acids					
		Total	Free		Total	Neutral fat				
		mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*m. eq. per 1000 cc.*	*m. eq. per 1000 cc.*	*mgm. per 100 cc. whole blood*	*grams per 100 cc. serum*		
Experiment I										
L	0	206	50	8.9	11.8	2.6	0.3	6.79		
	2	210	52	8.8	11.6	2.4	8.9	7.32	O	R.
	5	219	63	9.5	11.6	2.0	13.9	7.53	O	R
F	0	147	43	8.5	7.1*	−0.5*	0.3	6.56		
	2	129	44	8.6	8.3*	1.1*	7.2**	6.90	O	R
	5	209	58	9.5	9.8	0.4	14.8	7.34	O	R
Q	0	185	49	8.7	10.5	1.9	0.4	7.01		
	2	203	67	9.3	9.6	0.7	0.3	7.40	O	O
	5	203	56	8.3	9.3	0.7	2.5	7.61	O	A. L.
D	0	172*	42	7.6	12.5*	4.7*	0.3	7.10		
	2	174	56	8.0	8.9	1.2	16.6**	7.49	O	R
	5	171	48	8.2	11.7	3.7	23.9**	7.30	O	D. S.
P	0	192	61	8.9	12.6	4.0	0.3	6.79		
	2	190	63	9.0	11.7	3.2	2.3	7.48	C	R
	5	193	47	8.9	10.8	1.9	3.0	7.41	C	R
R	0	141*	33	6.5	6.2*	−0.4*	0.3	6.38		
	2	140	46	7.2	8.0	1.4	2.5	7.03	C	R
	5	146	42	7.5	8.3	1.2	3.2	7.46	C	R
W	0	170	52	9.1	9.7	1.4	1.1	6.63		
	2	177*	47	8.4	9.7	1.5*	1.9	7.28	C	R
	5	151	40	8.8	8.6	0.6	3.0	7.10	C	R
H	0	215*	56	8.5	11.4*	2.3*	0.3	6.97		
	2	213	60	8.5	10.0	1.1	2.5	7.37	C	R
	5	165	47	7.6	8.1	0.6	3.4	6.70	C	D. S.
Experiment II										
A, Q	0	205	57	10.4	11.6	1.7	0.2	7.33		
	2						16.6		O	O
	3	246	76	11.0	15.8	4.9	15.5	8.09	O	O
	4						3.1		C	A. L.
B, Q	3						0.6		F, C	A. F. J.
A, C	0	163	46	8.5	9.3	1.3	1.1	6.38		
	2						12.6		O	O
	3	197	56	9.0	11.3	2.4	9.8	7.35	O	O
	4						3.2		C	A. L.
B, C	3						6.1		F	A. F. J.
A, H	0	218	55	8.7	9.9	0.6	0.3	6.99		
	3	255	67	9.4	12.7	2.4	13.9	7.74	O	O
	4						2.4		C	A. L.
B, H	3						7.8		F	A. F. J.

TABLE I—*Continued*

Subject	Days	Serum lipids: Cholesterol, Total	Serum lipids: Cholesterol, Free	Serum lipids: Lipid phosphorus	Serum lipids: Fatty acids, Total	Serum lipids: Fatty acids, Neutral fat	Ketones as acetone	Total proteins	Food***	Fluid****
		mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*m. eq. per 1000 cc.*	*m. eq. per 1000 cc.*	*mgm. per 100 cc. whole blood*	*grams per 100 cc. serum*		
Experiment II—*Continued*										
A, T	0	172	43	9.6	10.1	1.2	0.3	7.32		
	3	185	55	9.6	12.7	3.8	3.7	7.88	O	O
	4						0.9			
B, T	0	156	44	9.2	11.3	3.1		6.68		
	3	149	39	8.4	11.6	3.9	4.7	7.68	O	A. F. J.
A, R	0	117	32	7.9	8.1	1.3	0.2	6.28		
	3	168	49	8.0	14.3	6.6	20.8	6.93	O	O
B, R	0	141	39	7.8	8.8	1.7		6.22		
	3	173	46	8.3	11.9	3.1	37.4	6.85	O	0.6% NaCl
Experiment III										
H	0	213	52	8.2	10.8	1.8	0.3	7.12		
	2	223	55	8.2	11.2	2.1	17.6	7.31	O	R
	4						4.5		F	O
	5						3.9		F	O
	6						2.9		F	O
	7						5.0		C	A. L.
C	0	195	52	8.2	9.4	1.0	0.6	7.07		
	2	203	55*	9.2*	10.9	1.7*	11.9	7.31	O	R
	4	230	64	10.0	11.6	1.5	17.7	7.79	O	O
	5	232	66	10.1	12.2	2.0	22.4	7.82	O	O
	6	236	65	10.1	12.6	2.4	33.0	7.70	O	O
	7	202	56	9.1	11.3	2.2	20.5	7.26	C	A. L.
T	0	200	55	11.7	15.7	5.2	0.3	7.23		
	2	196	54	10.9	13.3	3.3	3.0	7.49	O	R
	4	200	54	10.0	14.2	4.6	6.5	7.98	O	O
	5	203	55	9.7*	14.1	4.6*	6.6	7.76	O	A. L.
M	0	189	47	10.4	13.3	3.6	0.3	7.11		
	2	199	48	9.4	13.0	3.6	9.0	7.20	O	R
	4						11.1		F	A. L.
	5						25.8		O	A. L.
Experiment IV (Students)										
B	0	211	50	8.8	11.6	2.3	0.4			
	2	224	63	9.8	15.6	6.4	29.6		O	A. L.
Bl	0	232	60	10.5	18.2	7.6	0.2			
	2	243	62	10.2	17.0	6.4	12.5		O	A. L.
F	0	163	48	6.7	7.8	0.9	0.3			
	1						3.9		O	
	2	189	60	7.6	10.5	2.8	13.7		O	A. L.

* Single determination. All others average of duplicate determinations.
** Poor checks between duplicates.
*** In the food column, C stands for carbohydrate, F for fish in the previous period.
**** In the fluid column, R stands for restricted and A. L. for *ad libitum* water intake, D. S. for dilute seawater, and A. F. J. for artificial fish juice.

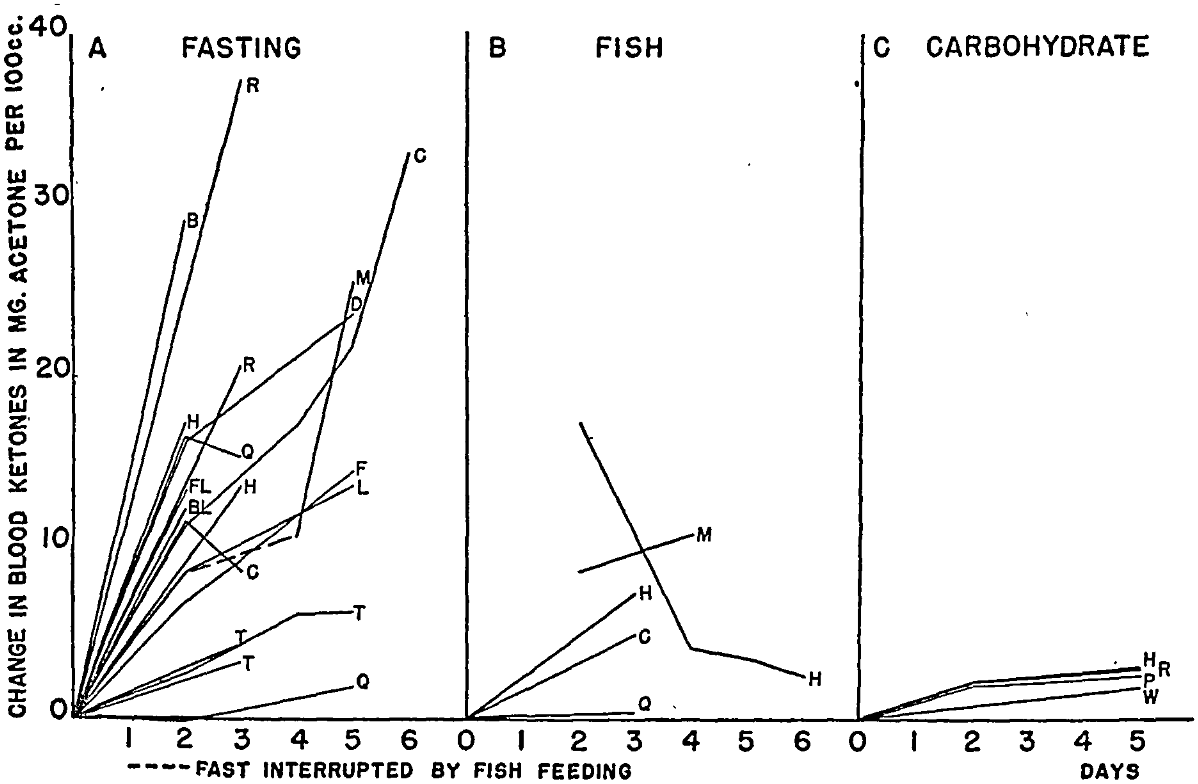


FIG. 2. BLOOD KETONE CHANGES IN HUMAN SUBJECTS
FIG. 2A. DURING STARVATION

The interrupted line indicates that the starvation was interrupted in one subject by feeding of fish for 2 days.

FIG. 2B. DURING FEEDING OF FISH

In the 2 instances in which there are no initial values, the subject has been fasted for 2 days prior to the feeding of fish.

FIG. 2C. DURING CARBOHYDRATE INGESTION

mgm. per cent, is relatively larger and more significant.[4]

Lipid phosphorus rose in all but 5 instances, exceeding the range of normal variability, ±0.7 mgm. per cent, on 7 occasions (see Figure 1). The average increase after 3 or more days was 0.8 ± 0.7 mgm. per cent.

The average increase of total fatty acids was 1.5 ± 2.2 m. eq. per liter; for periods of 3 days or more, it was 2.5 ± 1.9 m. eq. per liter. Changes of neutral fat were quite irregular. In several instances, it decreased. Only at the 3-day interval was it consistently elevated. In all of 5 determinations at this interval, neutral fat was definitely above its initial concentration.

[4] The normal average variation of free cholesterol has been calculated from Sperry's data (28). Free cholesterol was determined on 9 separate occasions in one subject and on 7 occasions in another. Some specimens were taken in the absorptive and others in the post-absorptive state. The average variation of one subject from his own average was 3 mgm. per cent, of the other 4 mgm. per cent.

Whole blood ketone bodies rose consistently and usually progressively during starvation (see Figure 2A). The average initial concentration in the 14 subjects was 0.4 mgm. per cent of acetone; after 2 days, it was 11.1 mgm. per cent. In most longer studies, it rose further from the second to the fifth day, reaching 37.4 mgm. per cent in Experiment II B–R after 3 days and 33.0 mgm. per cent in Experiment III C after 6 days. In no instance, however, did ketosis attain sufficient severity to produce a significant bicarbonate deficiency (16).

The degree of ketonemia varied from subject to subject and in different experiments on the same subject. There was no exact correlation between ketonemia and hyperlipemia. For example, the greatest ketonemia, 37.4 mgm. per cent, occurred in Experiment II B–R, in which cholesterol rose only 32 mgm. per cent; whereas a cholesterol increment of 41 mgm. per cent in Experiment II A–Q was associated with a blood

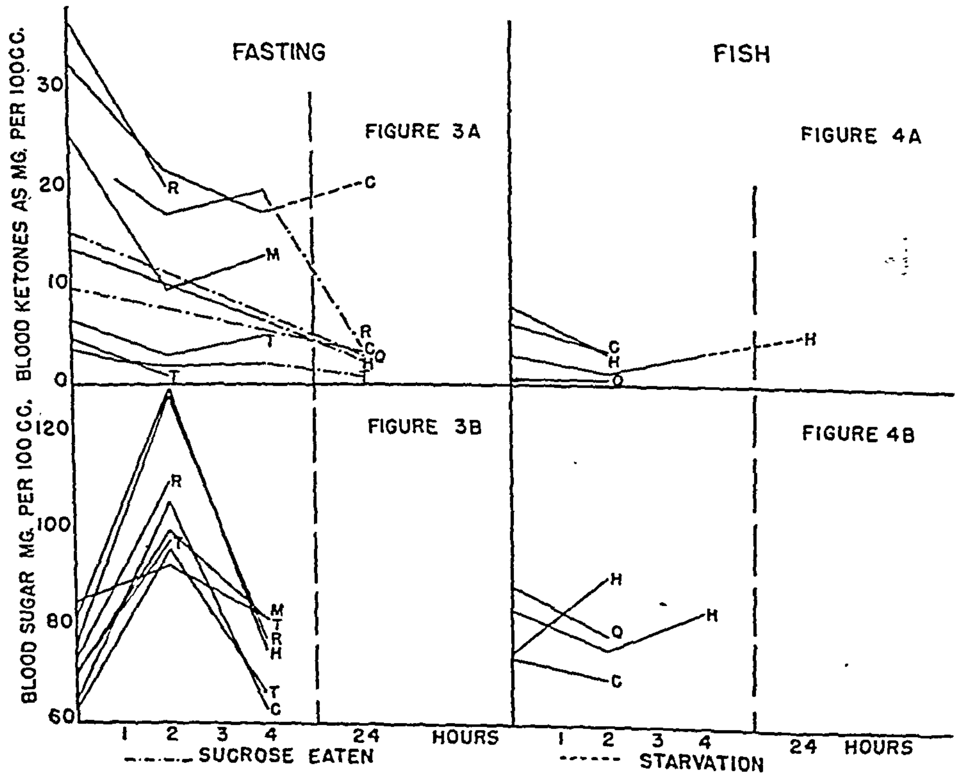
ketone of only 15.5 mgm. per cent. Nevertheless, a rough relation between the two functions is implicit in the fact that both rose progressively as starvation was prolonged.

None of the lipid fractions of the serum, except the lipid phosphorus of R, rose definitely in the 4 experiments in which subjects took 100 grams of sucrose daily (Experiment I, P, R, W, and H). In these experiments, blood ketones did not exceed 3.4 mgm. per cent, even after 5 days without other food (Figure 2 C), although 2 of the same subjects, H and R exhibited considerable ketonemia and increases of serum total cholesterol on starvation.

The intravenous injection of 25 grams of glucose at the close of starvation regularly and rapidly reduced the ketonemia of subjects who had starved or received fish (Figures 3A and 4A). Subsequent ingestion of carbohydrate during the remainder of the first 24 hours after starvation further depressed blood ketones, although they did not return to normal.

In 3 experiments in which fish was eaten for 3 days (Experiment II B–Q, C, and B), there was less ketonemia than in the comparable control experiments (II A–Q, C and H (see Figure 2B)). Of the 2 subjects who ate fish after a preliminary 2-day fast, only 1 (Experiment III–H) was able to eat considerable quantities. The ketones in this case diminished considerably, but did not return to normal.

Glucose tolerance of all subjects, except T, was distinctly low after 3 to 6 days of complete starvation, but was normal in subjects who had



Figs. 3 and 4. Changes in (A) Blood Ketones and (B) Blood Sugar Values, During and Following Intravenous Glucose and Feeding of Sucrose
Fig. 3. Following Starvation

Fig. 4. Following Experiments in Which Fish Had Been Eaten
Solid lines indicate changes following the intravenous injection of 25 grams of glucose at zero time. Dash line extensions indicate continued starvation during the next 24 hours. Dot-dash lines refer to experiments in which 100 to 150 grams of sucrose were ingested at intervals during the period indicated.

eaten fish (Figures 3B and 4B). No blood sugar below 64 mgm. per cent was found in any subject, even after 5 or 6 days of fasting.

B. Experiments with monkeys

Data from these experiments are given in Table II. Fasting invariably provoked striking hyperlipemia and ketonemia. The serum lipids were elevated at the end of 2 days and rose further subsequently. Cholesterol was most affected, increasing on the average 58 mgm. per cent, or 49 per cent of the initial value. The increment consisted chiefly of cholesterol esters; consequently, the ratio of free to total cholesterol fell. Lipid phosphorus rose proportionally less than cholesterol, an average of 2.4 mgm. per cent, or 30 per cent of the initial value. Neutral fat did not change consistently.

Blood ketones rose markedly, but to a variable extent, in all starved monkeys. The ketonemia

TABLE II
Serum lipids and blood ketones of monkeys

Monkey	Days	Serum lipids					Ketones as acetone*	Total proteins	
		Cholesterol		Lipid phosphorus	Fatty acids				
		Total	Free		Total	Neutral fat			
		mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*m. eq. per 1000 cc.*	*m. eq. per 1000 cc.*	*mgm. per 100 cc. whole blood*	*grams per 100 cc. serum*	
1545	0	105	24	7.8	7.8	1.4	1.1*	7.1	Control study, eating normally but in post-absorptive state
	3	155	30	10.7	10.7	0.8	12.9	7.7*	After 84 hours without food
2156	0	141	33	8.7	9.0	1.1		7.6	Control, eating normally, but in post-absorptive state
	5	134	30		8.9		0.9		
	0	157	45	8.8	9.0	1.0		7.2	
	2	181	46**		10.7		36.6*		After 2 days without food
	6	225	51	9.9	10.6	2.3	46.9	7.4	After 6 days without food
	0	132	25	8.4	6.9	−0.8	0.7		Control study, eating normally
	7	176	46	8.1	8.6	0.5	0.8		After 1 week of 15 grams of sucrose daily
	0	117	25	7.7	8.2	1.4	1.0*	6.2	Control, eating normally
	5	220	45	10.4	13.2	2.6	58.8	7.1*	After 5 days of starvation
	0	152	38	9.7	9.3	0.8	0.9*	6.6	Control, eating normally
	4	197	46	10.5	10.2	0.5	5.2	7.3	15 grams sucrose daily for 4 days
	8	155	39	8.5	9.6	0.6	0.8	7.2	40 grams sucrose daily for 4 days
	0	145	31	8.5	9.7	1.8	1.3	6.4	Control
	3	157	39	9.2	9.5	1.1	4.3	6.8	15 grams sucrose daily
	7						1.2		40 grams sucrose for 4 days
1739	0	113	30	8.4	7.3	0.3		6.6	Control, eating normally but in post-absorptive state
	0	119	29		6.9		0.8		
	0	119	32	7.2	6.5	0.1		6.7	
	2	172	40	10.7	9.5	−0.2	12.6	6.9	After 2 days without food
	6	189	46	9.2	8.8	−0.3	9.7	6.4	After 6 days without food
	0	124	29	7.5	6.6	−0.3	0.6		Control
	7	191	49	9.0	8.7	−0.2	1.4	6.6	After 1 week 15 grams sucrose daily
	0	162	40	9.2	8.7	0.2	0.7*	6.0	Control
	4	171	44	9.2	9.8	1.2	4.0	6.9	After 4 days 15 grams sucrose daily
1567	0	144	32	8.9	9.5	0.9	1.6	7.3	Control
	0	134	39	9.1	9.2	1.3	1.3		Control
	4	157	41	7.9	7.4	−0.1	2.8		After 4 days 20 grams sucrose daily
	7	107	31	7.4	7.7	1.5		7.2	After 3 days of 50 grams sucrose daily

* One determination.

** Hemolysis.

usually was greatest in the animals that starved longest. No definite quantitative correlation between hyperlipemia and ketonemia could be established in these experiments.

Ingestion of 15 to 20 grams of sugar daily for from 3 to 7 days prevented the rise of lipid phosphorus, but not of cholesterol, and diminished ketonemia. When 40 to 50 grams of sugar were given daily, ketonemia was abolished and serum cholesterol fell to or below its initial concentration. This is distinctly less than the daily amounts of carbohydrate given to monkeys in their regular diets.

C. *Experiments with dogs*

Seven dogs were starved for from 4 to 14 days. The data are not presented in detail because there were no consistent changes of the serum lipids. In one animal, after 14 days, blood ketones rose to 1.5 mgm. per cent.

DISCUSSION

It has been rather generally stated that starvation is attended by hyperlipemia in which neutral fat is particularly involved. Evidence for these statements is, however, largely inferential, based on studies of diabetes uncontrolled by insulin and on the well-established fact that the lipids of the liver increase in starvation. In the present studies, a slight, but significant, increase of serum lipids has been demonstrated in normal men during starvation. A more pronounced hyperlipemia was observed in monkeys. The serum lipids of the dog were unaffected by starvation. In all species, neutral fat was only slightly altered. In both man and monkey, the lipid increment consisted of cholesterol and phospholipid, the former predominating.

The human experiments are somewhat complicated by the presence of hemoconcentration which regularly attends starvation and was exaggerated in the majority of these experiments by water deprivation. It has been shown by Man and Peters (29) that, when acute hemoconcentration is induced by prolonged maintenance of the erect posture, serum lipids and proteins rise proportionally. Evidence has also been adduced that the hyperlipemia of diabetic acidosis may be referable in part to hemoconcentration (30). During recovery after the acute phase of this condition, serum lipids and proteins parallel one another in their descent. Hemoconcentration may have been responsible for some part, but not all, of the increases of lipids in the human starvation experiments. Cholesterol rose proportionally more than the serum proteins. In addition, ingestion of carbohydrate in Experiment I inhibited hyperlipemia without greatly mitigating dehydration, while the lipids of Br, Bl, and Fl (Experiment IV) rose, although the subjects were allowed to drink water *ad libitum*. In the monkeys, lipids rose so much more than proteins that the reality of the hyperlipemia cannot be questioned.

The general concept that the hyperlipemia of starvation arises merely from the rapid mobilization of fat seems hardly tenable in view of these observations. Already there is a wealth of evidence that the concentrations of lipids in the serum are little influenced by the quantity of fat in the metabolism mixture. After fatty meals, there is a transient hyperlipemia, affecting chiefly neutral fat (31 to 36). On the other hand, the serum lipids—and especially cholesterol—of a given individual remain remarkably constant throughout a day and over long periods despite variations of diet (24, 25, 34, 35, 37 to 39). Starvation lipemia must, therefore, be attributed to a change in the character, rather than the quantity of fat metabolism.

The most obvious phenomenon with which to connect it is ketosis. The dog, inured to a carbohydrate-free diet, does not change the character of its metabolism radically with starvation. It also develops neither ketosis (10) nor lipemia (1 to 5, 8, 9). In the diabetic dog, however, both ketosis (40 to 42) and lipemia (43 to 47) occur. The human male, when starved, exhibits mild ketosis and a comparably slight lipemia. The two phenomena tend to parallel one another. The monkey is somewhat more susceptible to ketosis (42, 48) and has a proportionally greater lipemia.

In both man and monkey, lipemia can be abolished by the administration of quantities of carbohydrate altogether too small to alter radically the quantities of fat oxidized, but large enough to reduce ketosis to minimal proportions. Reduction of ketosis by administration of carbohydrate has been repeatedly reported (11, 15, 41).

Transfer from a mixed diet to 100 grams of carbohydrate, in the case of an adult male, for example, which must involve the consumption of at least 1200 additional Calories from fat per day, had no appreciable effect on serum lipids. Nevertheless, removal of 400 Calories in the form of sugar, thereby provoking ketosis, elicited distinct hyperlipemia. The fatty meals given by Man and Gildea (36) contained as much as 2500 Calories of fat. In the monkey, also, amounts of carbohydrate too small to reduce considerably the quantities of fat metabolized, but large enough to mitigate ketosis, reduced hyperlipemia.

Greater ketosis and lipemia might have been demonstrable in women and in children than in adult males. Deuel and Gulick found greater ketonemia in fasting women than in men (49). McQuarrie, Husted, and Bloor (50) have reported striking elevations of cholesterol, phospholipids, and fatty acids in the serum of epileptic children receiving ketogenic diets. The lipids fell when enough carbohydrate was given to eliminate ketosis. Hypercholesterolemia has also been reported by Tolstoi and his associates (51, 52) in an adult male who subsisted for prolonged periods on diets consisting solely of fat and protein. This is at variance with the report of Corcoran and Rabinowitch (53) of diminished rather than elevated serum cholesterol and phospholipids in Eskimos. The latter, however, did not have ketonemia, while Tolstoi's subjects did.

In the acidosis of human diabetes, in which both ketosis and hyperlipemia attain a severity never reached in starvation, neutral fat is usually affected as much or more than are cholesterol and lipid phosphorus (30). In this respect, for some reason, the lipemia differs from that of starvation. The distinction is not, however, an absolute one. Although cholesterol and lipid phosphorus are always elevated, neutral fat sometimes escapes. This is illustrated by comparison of the first 3 and last 3 cases in Table III (cases and treatment described previously (30)). What determines the participation of neutral fat is not clear. It may be the severity of the ketosis or the nutritive state of the patient; but it is impossible to establish a clear correlation with either of these features from the data now available. The consistent rises of cholesterol and lipid phosphorus, in contrast to the capricious action of neutral fat, are common to both starvation and diabetic acidosis.

TABLE III

Serum lipids in diabetic acidosis of humans

Case number, sex	Days, Hours	Blood sugar	Serum: Carbon dioxide	Serum: Cholesterol	Serum: Lipid phosphorus	Serum: Fatty acids of neutral fat
		per 100 cc.	*volumes per cent*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*m. eq. per 1000 cc.*
A700		686		673	18.9	8.2
	3	480		680	16.3	5.6
F	1, 11	375		456	12.7	6.4
	3, 11	530		559	13.3	4.3
	5, 11	360		503	12.8	5.7
A30940		1080	26.9	157	8.8	3.1
F	7	94	46.4	110	5.7	3.1
A30929		649	27.1	454	13.4	3.9
F	12	474		452	12.8	4.4
A25652		418	11.6	490	19.9	14.4
M	3	492		212	10.6	12.0
A5815		713	11.4	304	20.9	30.1
	5	476	29.3	281	14.5	12.0
	9½	168	41.6	213	10.0	8.2
F	14	60	42.7	208	9.5	5.5
	1	267	44.9	203	10.1	6.3
	2	486	43.2	190	9.5	7.2
	11	275		209	11.4	7.1
29923		716	11.7	233	16.3	32.3
F	1		32.1	161	8.5	4.2
	2		34.5	117	6.2	4.0

The lipid disturbances of acute starvation contrast sharply with those of chronic undernutrition. In the latter, the serum lipids are characteristically reduced (54, 55). Administration of carbohydrate in starvation causes a rapid decline of the lipids; during recovery from malnutrition, the lipids rise from subnormal to normal concentrations. Nevertheless, there is reason to believe that starvation will cause the lipids of a chronically malnourished patient to rise as do those of normal subjects. During acidosis in malnourished diabetics, Man and Peters (30) observed normal or high concentrations of lipids that fell below normal on recovery. An illustration is found in Case A30940 of Table III. The course of the serum lipids during starvation in a malnourished patient without diabetes is illustrated in Figure 5. The woman had an inoperable gastric carcinoma causing pyloric obstruction. On admission, she had taken little food for many

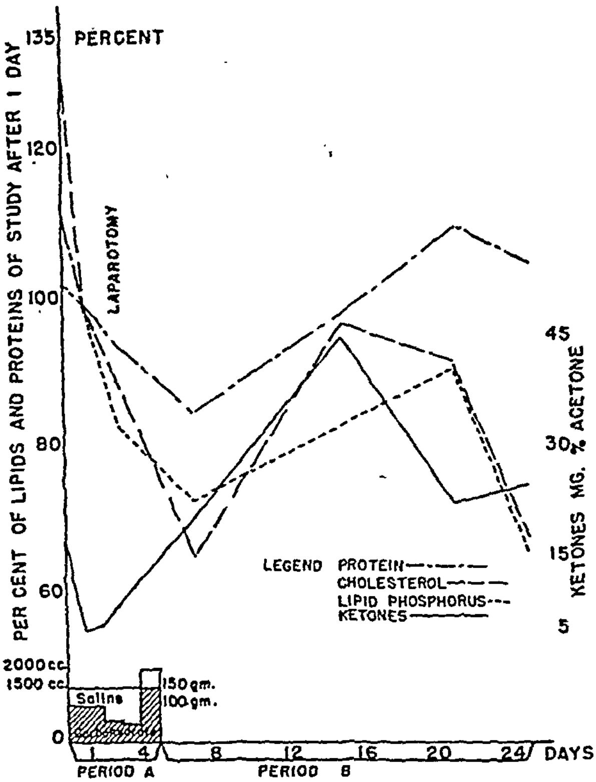


FIG. 5. BLOOD KETONES AND SERUM PROTEINS, LIPID PHOSPHORUS AND TOTAL CHOLESTEROL OF A PATIENT WITH AN INOPERABLE, OBSTRUCTING GASTRIC CARCINOMA

To represent proportionately the changes in serum lipid fractions and proteins, the values on the first morning after parenteral fluid and glucose have been selected as 100 per cent. These relative values can be translated into absolute terms from the following data: at 100 per cent, protein equals 5.58 per cent, cholesterol 163 mgm. per cent, and lipid phosphorus 10.4 mgm. per cent. Blood ketones are presented in absolute terms.

weeks and had vomited everything for 4 days or more. During the first few days (period A of Figure 5), while she received parenteral saline and glucose, all the serum lipid fractions fell considerably and progressively. Cholesterol diminished more than lipid phosphorus did, finally falling below the normal range. Serum proteins also declined, but relatively less than the lipids. Blood ketones diminished within 24 hours, but never to normal values. With resumption of starvation (period B), blood ketones and lipids again rose for a time. The initial study in period B is difficult to evaluate because no blood study was obtained soon after carbohydrate administration was stopped. As starvation was prolonged and wasting became extreme, both ketones and lipids gradually decreased again.

These experiments and the other scanty data which can be found in the literature strongly suggest that the hyperlipemia of carbohydrate starvation is not simply an indication that fat is being burned more rapidly, but that a larger proportion of fatty acids is being converted to ketone bodies in the process of combustion. There is ample evidence that the quantity of fat in the metabolism mixture can be varied greatly without any demonstrable variation of serum lipids so long as it is burned directly by the tissues and no unusual amounts of ketone bodies are formed. When, however, ketogenesis exceeds certain limits, hyperlipemia appears. In this, cholesterol and phospholipids seem to play a major role, presumably serving as vehicles to convey the fatty acids to the liver. It may be that they are required to facilitate the entrance of the fatty acids into the liver and participate in the reactions by which ketone bodies are formed. It is well recognized that the hepatic metabolism of fat is greatly influenced by phospholipids and cholesterol.

SUMMARY AND CONCLUSIONS

A study has been made of the serum lipids and blood ketones of adult human males, dogs, and monkeys during starvation.

Dogs developed no appreciable ketosis and no hyperlipemia.

In men, blood ketones increased progressively throughout the periods of starvation, which varied from 2 to 6 days. Serum cholesterol rose slightly, but unequivocally, as fasting was prolonged; lipid phosphorus rose perceptibly; neutral fat changes were equivocal.

In monkeys both blood ketones and serum lipids rose more rapidly and further than they did in men. Again cholesterol was most affected, while neutral fat did not change appreciably.

In both man and monkey, the hyperlipemia was abolished by administration of sufficient carbohydrate to mitigate or to extinguish the ketosis.

From this and other evidence, it is suggested that the hyperlipemia of carbohydrate starvation arises not merely because a larger quantity of

fat is being utilized, but because an unusually large amount of fat is being converted to ketone bodies.

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HEPATITIS DUE TO THE INJECTION OF HOMOLOGOUS BLOOD PRODUCTS IN HUMAN VOLUNTEERS[1]

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It is now recognized that human whole blood, plasma, or serum may occasionally contain an agent, or agents, capable of producing hepatocellular jaundice when administered parenterally to susceptible persons (1 to 4). Thus far, the only method of detecting the presence of this agent in such materials is by testing them in human volunteers. The present studies in human volunteers, which were initiated by the occurrence of jaundice in a case previously described (3), have yielded further information concerning certain aspects of this disease. This report presents the methods employed and the initial results obtained in the study of 9 additional cases of experimentally induced hepatitis. The literature pertaining to serum jaundice has been adequately reviewed elsewhere (1 to 3).

METHODS

Volunteers. Nine white male volunteers between the ages of 18 and 25 were selected on the basis of the following criteria: (*a*) No significant medical history or physical abnormalities. (*b*) Normal blood count, urinalysis, serological test for syphilis, and erythrocyte fragility. (*c*) Normally functioning gall bladder as indicated by cholecystography. (*d*) No evidence of hepatic disturbance detectable by a group of liver function studies. During the period of investigation, the volunteers acted as attendants at a nearby hospital where their duties were mildly to moderately strenuous. The diet consisted of the ordinary hospital fare and seemed to be adequate. Risk of an epidemic of jaundice as a result of contact with the volunteers was considered minimal because of the apparent absence of contact infection in the jaundice outbreak (U. S. Army 1942) related to the serum component of yellow fever vaccine (4). A few cases of jaundice had occurred among the patients of this institution during the year before this study was begun, but no case was subsequently observed, among the hospital population of approximately 6000 patients and 590 employees, until after the experimentally induced disease had appeared in the volunteer group, when 2 of the employees developed jaundice (see below).

One of the volunteers (H. J. C.) had a history of jaundice 4 years prior to this study, but the other 8 had no history of jaundice. Several had attended patients with jaundice during the preceding year. The volunteers were observed for 1 to 3 weeks before inoculation and none showed evidence of hepatic dysfunction during this period.

Procedure. After obtaining a specimen of plasma for preservation in the frozen state, the volunteers were inoculated with one of several suspected materials in order to determine the presence of an icterogenic agent. They were then studied, at frequent intervals, by means of a group of liver function tests so as to detect the earliest evidence of hepatic disturbance. As soon as this appeared, the men were hospitalized.[4] Plasma or serum, stools, bile, nasopharyngeal washings, and urine, to be used in future experiments, were collected during the pre-icteric and icteric stages.

The materials used for inoculation were as follows: 1. Plasma A—pooled convalescent mumps plasma, obtained in April 1942 from a group of 14 soldiers of the U. S. Army, stationed in this country, during their convalescence from mumps. The blood was collected in bottles containing sodium citrate as an anticoagulant; the plasma was removed, cultured, and frozen. The routine cultures were negative. In June 1942, a civilian with mumps was treated with this plasma and 76 days later developed jaundice (3). The plasma was considered responsible for transmission of the icterogenic agent and was withheld from further usage. When used in this study, it had been preserved in the frozen state for approximately 18 months. Previous studies had shown that this material was not icterogenic for a large number of animals (5, 6). *2. Plasma B*—obtained from volunteer B. C. during mild hepatitis, 23 days after his inoculation with plasma A. The blood was collected in a bottle containing sodium citrate as an anticoagulant; the plasma was removed, cultured, and frozen. The cultures were negative. The plasma was used in this study 1 and 2 months after collection. *3. Yellow fever vaccine (lot No. 335)*—This vaccine was one of the lots associated with the occurrence of jaundice in the U. S. Army in 1942 (4). The pooled human serum in this vaccine has been regarded as the

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carrier of the icterogenic agent. This vaccine was prepared in November 1941 and was dispensed under vacuum, in the desiccated state in sealed ampoules. It had been kept at a low temperature until June 1942, but from that time until used in this study in November 1943, it had been kept at room temperature in Washington, D. C. It was prepared for injection in accordance with the standard directions, *i.e.*, the suspension of dried contents of 1 ampoule in 55 ml. of physiological salt solution. In March 1944, intracerebral inoculation of mice showed that the yellow fever virus in this vaccine was no longer capable of producing the disease.[5]

Special studies. (Normal ranges listed herein are based on the preinoculation studies *of the inoculated group.*) *A. Blood: The serum bilirubin concentration* was measured by the method of Malloy and Evelyn (7). The normal range for this group was found to be between 0.2 and 1.4 mgm. per 100 ml. and the level in the same normal person occasionally varied as much as 0.7 mgm. *The bilirubin excretion test* (8) was used initially because of its reputed value as one of the most sensitive indicators of hepatic dysfunction in the absence of jaundice. The results, to be reported elsewhere, were inconsistent and apparently subject to random influences to such an extent that the test was discontinued. *The Van den Bergh reaction* was determined by the ring test. *The cephalin-cholesterol flocculation test* was performed according to the technic of Hanger (9). While valuable information was obtained by this test, the results could not be accepted with the same assurance as those of certain other tests, owing to the frequent occurrence of 1 and 2 plus reactions in normals. Readings at 48 hours presented greater difficulties in this respect than did those at 24 hours; the results shown, therefore, are the 24-hour readings. In addition to this, technical difficulties have lowered our confidence in the procedure. Differences in sensitivity of the antigen extend to vials bearing the same lot number and purchased at the same time. Duplicate tests set up in different laboratories by careful and experienced workers, using the same reagents, often failed to agree completely.[6] It is evident that the procedure must be standardized with considerable care and controlled by frequent determinations on normal sera. If this is done, the test is capable of providing helpful data. *The fractional cephalin-cholesterol flocculation test* proposed by Bruger (10) gave abnormal results in some dilutions in all normal controls, and was therefore abandoned. Mirsky and Von Brecht (11) have reported similar experiences with this fractional test. *Serum total protein, albumin, and globulin concentrations* were determined by the biuret photoelectric method of Kingsley (12). The preinoculation range for this group was: Total protein 6.9 to 8.2; albumin 5.0 to 6.0; globulin 1.6 to 2.6 grams per 100 ml. *Total and esterified serum cholesterol* levels were estimated by the method of Reinhold (13) and the range was: Total—158 to 286 mgm. per 100 ml., esters—95 to 161 mgm. per 100 ml., or 50 to 76 per cent of the total. *Serum alkaline phosphatase* was measured by the method of Bodansky adapted to the photoelectric colorimeter (14). The range before inoculation was from 1.5 to 3.4 Bodansky units. *Prothrombin activity* was determined by the method of Quick (15). Before inoculation it varied between 70 and 100 per cent of normal. *Vitamin A*[7] was estimated in blood plasma by the method of Kimble (16) which is based on the Carr-Price reaction. The results have been expressed in International Units, calculated according to Kimble's directions. Facilities for this determination were not available at the beginning of the investigation, so that preinoculation levels could not be established. However, estimations were made in several cases before the onset of hepatitis and these results probably approach the preinoculation level. *Sedimentation rates* were determined by the Wintrobe technic (17). The range before inoculation was from 3 to 15 mm. per hour. *Erythrocyte fragility* was measured by the method of Waugh and Asherman (18). The procedure proved to be satisfactory, but the solutions were found to change in pH on standing, so that considerable time and effort were expended on the necessarily frequent checks of the reagents. *Routine blood counts* were performed periodically throughout the study. "*Target*" *cells* (19) were sought in the ordinary blood smears. *Bromsulphalein test*—the dose used was 5 mgm. per kgm. and the amount of retention was measured 30 minutes after the injection. The retention in this group before inoculation never exceeded 5 per cent. *B. Urine tests:* The diazo spot test of Godfried and the Harrison spot test (Godfried modification) (20) were employed routinely for the detection of *bilirubin in the urine.* The methods were quite satisfactory, although weakly positive results were infrequently obtained in normal individuals. In such cases, however, subsequent tests were negative. The methylene blue test of Franke (21) was discarded because of the frequent occurrence of false positive reactions. The "foam test" for bilirubin was quite insensitive compared to the diazo and Harrison spot tests. *Urobilinogen*—the test of Wallace and Diamond (22) was used routinely and none of this group had a positive test in the 1 : 10 dilution before inoculation. The method of Watson (23) was used at less frequent intervals because of difficulties in the collection of the required 24-hour urine specimens. *Bile acids* were sought by the method of Hay (24). It was realized that a negative test did not exclude the presence of bile acids. *Routine urinalyses* were performed periodically throughout the investigation. *The intravenous hippuric acid test* was performed according to the technic recommended by Quick (15). The dose

[5] Courtesy of Dr. Werner Henle, Assistant Professor of Bacteriology in Pediatrics, School of Medicine, University of Pennsylvania.

[6] Recent studies have shown that discrepancies obtained in the results of tests set up in 2 different laboratories are due, in large part, to variation in the amount of light to which the serum-antigen mixtures are exposed. When kept in the dark or in subdued light, the results are more uniform and fewer false positives are obtained (Science, 1944, 100, 83).

[7] Courtesy of Dr. Charles Urbach, Children's Hospital; Associate in Pediatrics, School of Medicine, University of Pennsylvania.

was 1.77 grams of sodium benzoate. Just before the injection, 400 ml. of water were ingested. The preinoculation range for the group was from 0.64 to 0.94 grams of benzoic acid excreted in the urine as hippuric acid in 1 hour. *C. Miscellaneous tests: A cholecystogram* was obtained on each volunteer before inoculation. The oral methods involving the use of either Priodax or tetraiodophenolphthalein were employed. Serologic tests included those for syphilis, typhoid, paratyphoid A and B, brucellosis, the Weil-Felix reaction, and heterophile antibodies.[8] Serologic studies for leptospira icterohemorrhagica were negative.[9] *Duodenal drainage* was carried out in a few cases during the icteric stage according to the usual technic. *The histamine test* of Klein (25) was frequently employed. It was inferior to the determination of serum bilirubin concentration, but would undoubtedly be helpful where this procedure is not available. *The water excretion test* was performed according to the technic suggested by Lichtman (26); namely, by measurement of the hourly urinary output for 4 hours after the ingestion of 1000 ml. of water. A normal adult should excrete the ingested amount within the 4-hour period. It was found, however, that a number of normals excreted abnormal amounts according to this interpretation (473 to 1290 ml.). The test was therefore abandoned.

Interpretation of results of special studies. The result of any one test was considered abnormal when it deviated beyond the preinoculation range of the group. In a few cases, results not beyond the range of the group, but deviating significantly from many determinations before and after inoculation in the same subject, were regarded as abnormal. As temporarily abnormal results with single tests were occasionally observed in the control group, hepatic disturbance was not considered to be present in the inoculated men unless *more than one* test was abnormal.

Control studies. In order to determine the responses of uninoculated normal persons to the repeated performance of a group of liver function tests, studies were made on 10 men who were quartered with the volunteers, 10 men living elsewhere, and on a laboratory technician connected with this investigation. Special effort was also made to carry out such studies on the controls during incidental minor illnesses, *i.e.*, upper respiratory infections, gastrointestinal upsets, and "grippe." The tests included, in all cases, determinations of serum bilirubin, urine bilirubin, and cephalin flocculation; in some cases, bromsulphalein excretion, serum protein, plasma vitamin A, prothrombin activity, and total and esterified cholesterol were also determined. It was not possible to study all of the control group as frequently or completely as was done in the case of the volunteers. However, several of the controls were studied at frequent intervals over periods of 4 to 10 weeks.

RESULTS

Incidence of hepatitis. The incidence of hepatitis following the inoculation of 9 human volunteers is shown in Table I. To the first group of 5 volunteers, who were inoculated intravenously with varying amounts of Plasma A, has been added the case of the patient (3) who received this plasma as an experimental therapeutic measure in 1942, making a total of 6. Of these, 5 developed hepatitis with jaundice. The remaining one (W. D. S., Figure 5) did not have indisputable evidence of hepatitis. However, he did have several brief episodes characterized by gastrointestinal symptoms and slightly abnormal results with the tests used to detect hepatic disturbance. Because these abnormalities were similar to those experienced by others of the group who later developed hepatitis, this case has been classified as probable mild hepatitis without jaundice. Two volunteers were inoculated intravenously with Plasma B. Both developed symptoms and laboratory manifestations of mild hepatitis without jaundice. Two volunteers were inoculated subcutaneously with 2 ml. of the yellow fever vaccine. One developed hepatitis with jaundice, and the other, hepatitis without overt jaundice.[10] The incidence of successful experimental transmissions of this disease was, therefore, 100 per cent. In an attempt to determine if immunity was conferred by 1 attack of this disease, 1 volunteer (R. R. M.) was reinoculated, with the same dose of the same material by the same route, 1 month after all signs of

TABLE I

Results of inoculation of human volunteers

Icterogenic material	No. inoc.	Hepatitis with overt jaundice	Hepatitis without overt jaundice		Negative results
			Definite	Probable	
Plasma A	6	5		1	0
Plasma B	2		2		0
Yellow fever vaccine, lot 335	2	1	1		0

[8] We are indebted to the William Pepper Laboratory of Clinical Medicine of the University of Pennsylvania for these studies.

[9] Agglutination tests were done through the courtesy of Dr. E. L. Stubbs, Professor of Veterinary Pathology of the University of Pennsylvania; complement-fixation tests by Dr. F. Boerner, Associate Professor of Clinical Bacteriology, Graduate School of Medicine, University of Pennsylvania.

[10] Serum obtained from both men 3 weeks following inoculation and examined by Dr. Max Theiler showed no neutralizing antibodies against the yellow fever virus.

the previous hepatitis had disappeared. During the 140 days that have elapsed since the second inoculation, no signs of hepatitis have been detected. Additional experiments of this type are now in progress.

Course of disease. The course of the disease in each of the volunteers is portrayed by the data charted in Figures 1 to 9. Mild symptoms and signs, usually occurring from 12 to 35 days after inoculation, marked the onset of the disease. This was followed by an asymptomatic interval or by periodic recurrences of mild symptoms and signs which, after 73 to 110 days, usually culminated in the familiar clinical picture of an acute hepatitis with jaundice. The course thereafter was similar to that observed in mild infective hepatitis.[11] For this reason, the main interest has centered about the long interval between inoculation and jaundice. As no evidence of hepatic dysfunction was detected in any of the volunteers prior to inoculation, abnormal results with more than one liver function test have been regarded as an indication of hepatic disturbance due to the inoculation. In this report, such a disturbance is referred to as hepatitis, since studies have shown that the pathologic picture, at least during the icteric stage, is that of hepatitis (27, 28). When interpreted on this basis, the charts show that hepatitis was present long before it was manifested by jaundice. In some cases, the process seemed to be active continuously during the interval between the onset of the disease and the development of jaundice, whereas in others, it appeared to be active intermittently. For purposes of discussion, it has been convenient to consider the course of the disease in 2 periods; an *early period*, which includes the first 60 days after inoculation, and a *late period*, which covers the interval beyond 60 days. Based on this arbitrary division, Table II shows the time of onset, in each volunteer, of the early and late manifestations of hepatitis. From these data, it is seen that the onset occurred during the first

TABLE II

The time of onset of the early and late manifestations of hepatitis

Subject	Inoculum	Dose and route	Hepatitis: Early period 0 to 60 days — Without jaundice	Hepatitis: Late period after 60 days — With jaundice	Hepatitis: Late period after 60 days — Without jaundice
		ml.	*days after inoculation*		
B. C.	Plasma A	1 (I-V)[1]	23	110	
C. R. L.	Plasma A	9 (I-V)	35	99	
F. S.	Plasma A	10 (I-V)	50	74	
R. R. M.	Plasma A	12 (I-V)		73	
W. D. S.	Plasma A	100 (I-V)	23[2]		130[2]
J. C.	Plasma B	11 (I-V)	12		
N. H. H.	Plasma B	20 (I-V)	23		85
H. J. C.	Y.F.V. 335	2 (H)[1]	28		65
S. B. E.	Y.F.V. 335	2 (H)	28	99	
Totals—9 cases			8	5	3

[1] (I-V)—Intravenous; (H)—Subcutaneous.
[2] Slight abnormalities have been regarded as probable hepatitis.

50 days in 8 of the 9 cases, and in 7 of these, it appeared between the 12th and 35th days. In 1 man (J. C.), manifestations of the disease were observed in only the early period; in 7, however, they occurred during both the early and late periods. In only 1 case (R. R. M.) did jaundice occur during the late period without preceding signs of hepatitis during the early period. In 4 cases (F. S., C. R. L., S. B. E., B. C.), jaundice did not occur for 24, 69, 71, and 87 days, respectively, after the first evidences of the disease had been detected. Furthermore, overt jaundice did not appear in 4 cases (W. D. S., J. C., N. H. H., H. J. C.) during either the early or late periods, although 3 of them had definite, and the other suggestive, evidence of hepatitis in one or both of the periods. These cases clearly demonstrate the occurrence of hepatitis without jaundice, a possibility which has been previously suggested by others (1, 29 to 32). That the appearance of hepatitis during the early period has not been confined to our series alone is suggested by 2 cases, previously reported (2), in which hyperbilirubinemia was first observed 3 and 5 weeks, respectively, after inoculation, and by the quoted statement of one of Cameron's volunteers (30):

[11] The term infective hepatitis is used here to include both the sporadic and the epidemic types of infective hepatitis. So-called sporadic and epidemic catarrhal jaundice, epidemic hepatitis, and infectious hepatitis or jaundice are other terms which are used in the literature more or less synonymously with infective hepatitis.

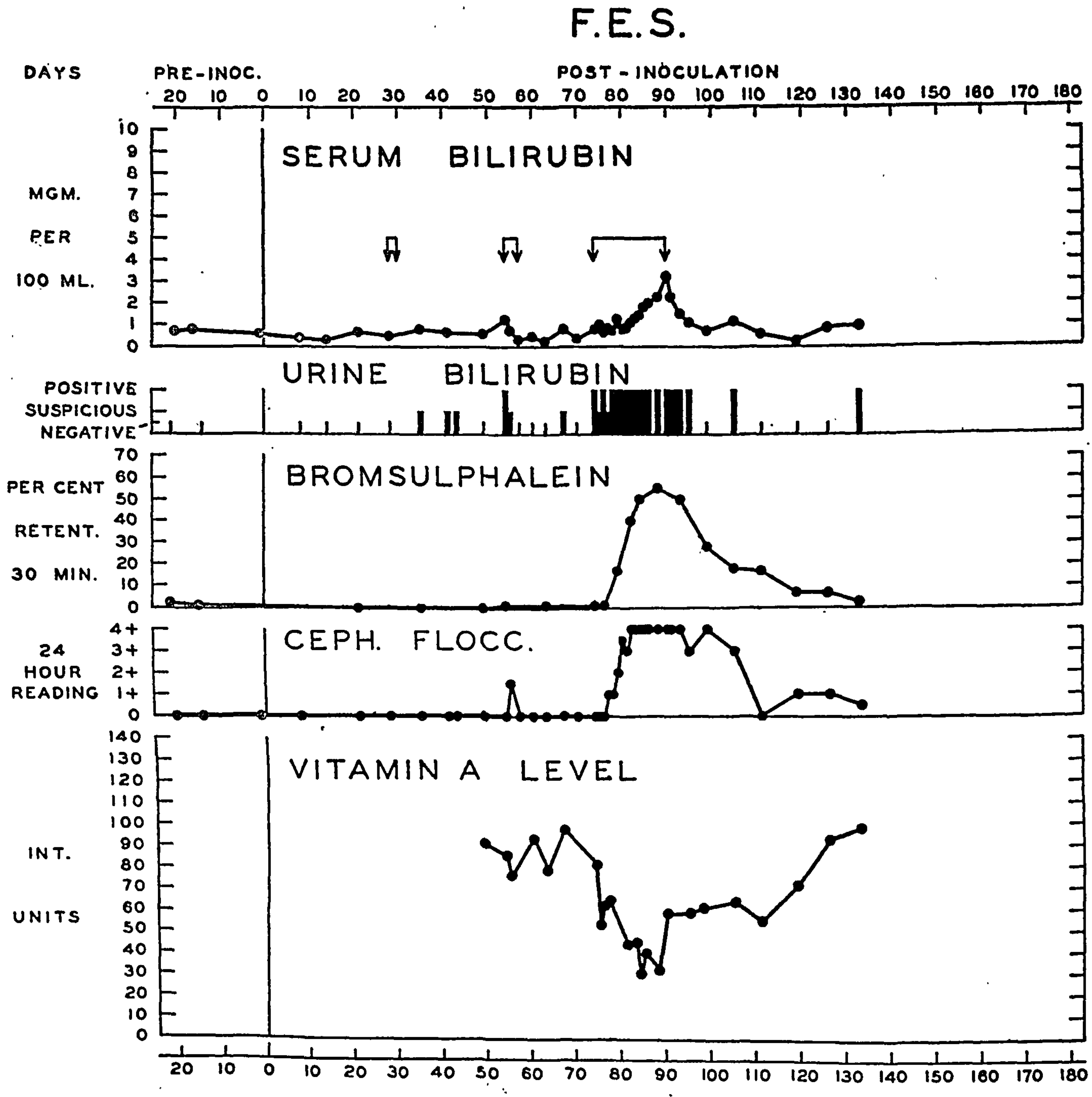

FIG. 3. STUDIES BEFORE AND AFTER THE INTRAVENOUS INOCULATION OF F. E. S. WITH 10.0 ML. OF PLASMA A

"A month after the injection I felt out of sorts and passed dark urine. I saw the regimental medical officer, but there was no need for hospital. I stuck it out well during the hard marches of the campaign, but just when it was over (nearly 6 months after inoculation) I went and got jaundice." It is apparent from these results that the onset of the disease is probably much earlier than has been recognized. Previous estimations of the incubation period have been based on the inoculation-to-jaundice interval, which has commonly been 60 to 100 days (1, 2, 33). On that basis, most of our cases had a similar incubation period. However, since the incubation period is defined as the interval between the entrance of an infective agent and the first manifestations of the disease (34), the true incubation period in most of our cases was 12 to

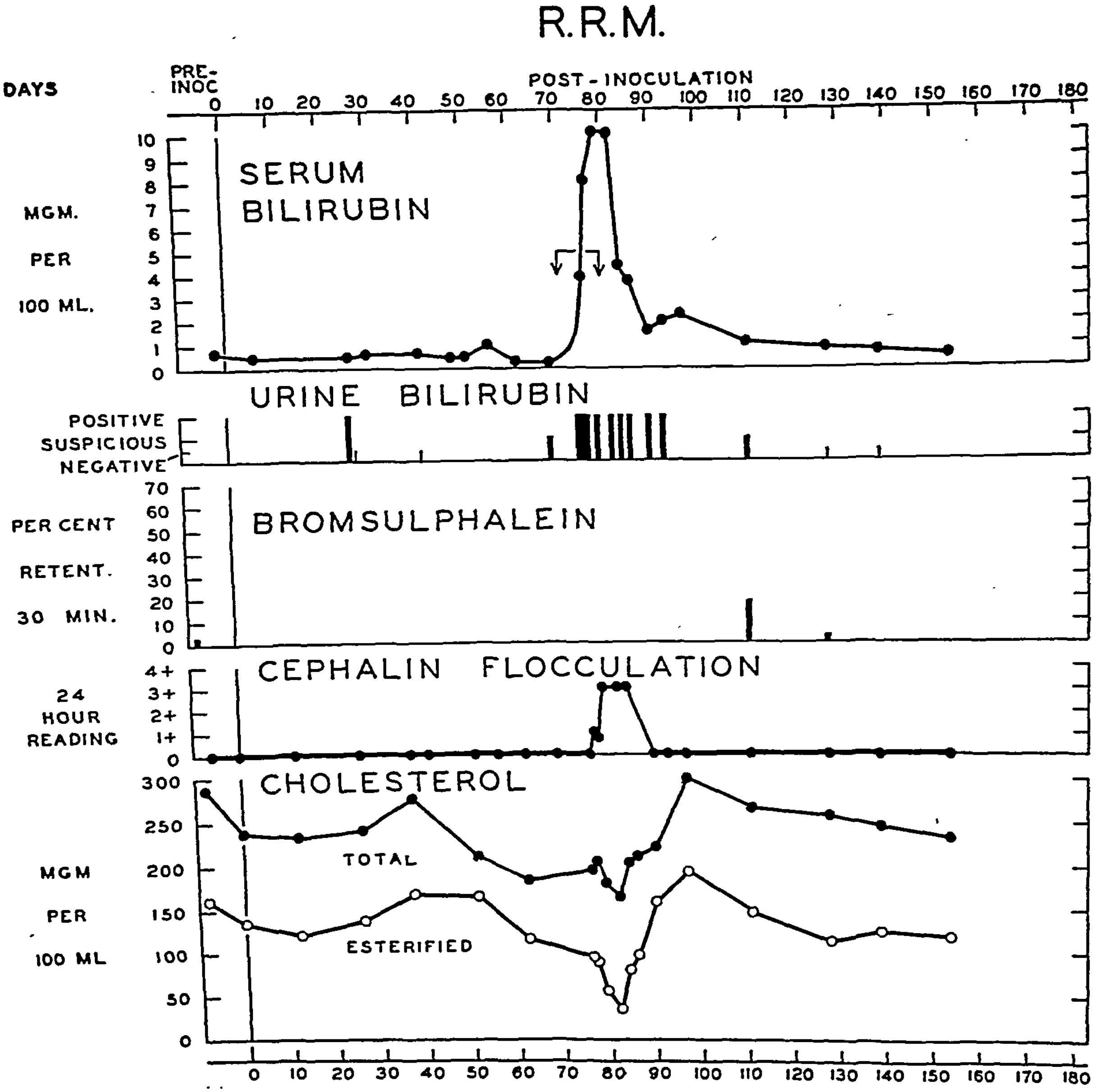

FIG. 4. STUDIES BEFORE AND AFTER THE INTRAVENOUS INOCULATION OF R. R. M. WITH 12.0 ML. OF PLASMA A

As only 3 bromsulphalein tests were performed, columnar charting has been used. Vitamin A determinations were not done on R. R. M.

35 days, an interval which approximates the incubation period of those forms of infective hepatitis unrelated to serum.

The symptoms and signs of the hepatitis that occurred during the *early period* were variable. The symptoms included malaise, weakness, anorexia, nausea, vomiting, muscular pains, and headache. Examination showed no consistent findings and jaundice was never observed. Slight fever was occasionally present but the temperature reached 102° F. in only 1 case (B. C.). Tenderness could usually be elicited on first percussion over the liver, but its edge was seldom palpable during this stage. The spleen was palpable in only 1 case (B. C.). These symptoms and signs usually disappeared after 1 to

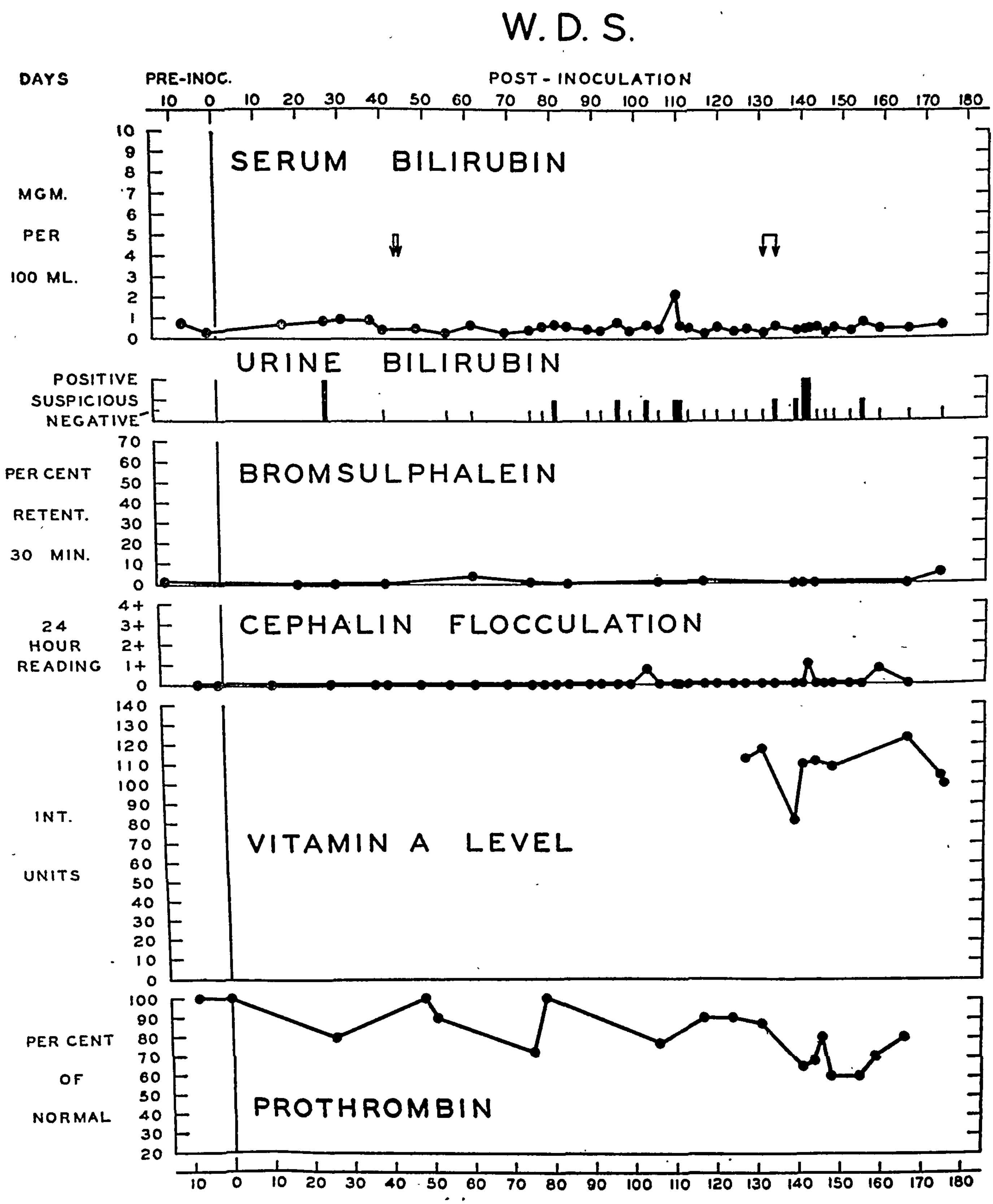

FIG. 5. STUDIES BEFORE AND AFTER THE INTRAVENOUS INOCULATION OF W. D. S. WITH 100.0 ML. OF PLASMA A

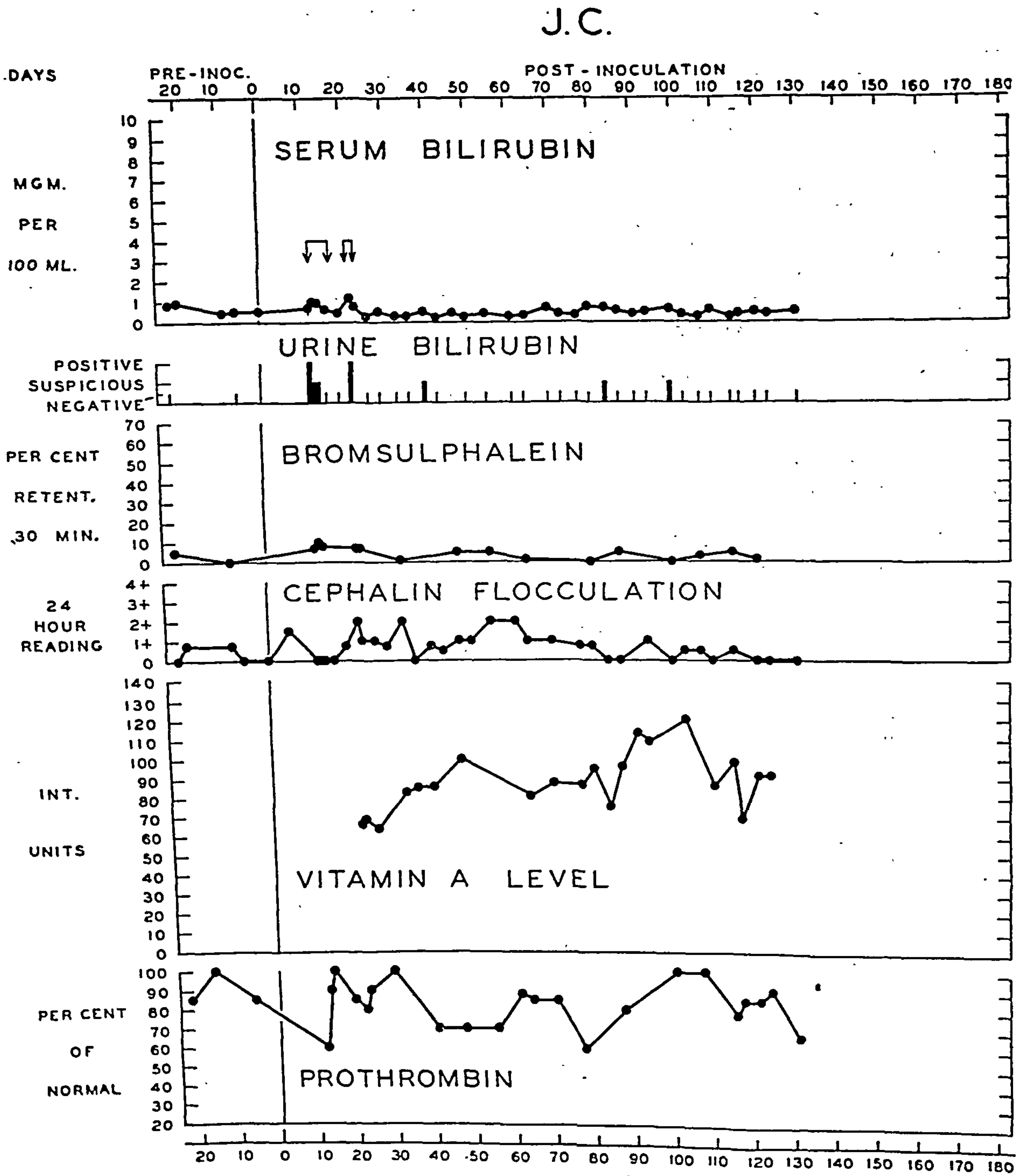

FIG. 6. STUDIES BEFORE AND AFTER THE INTRAVENOUS INOCULATION OF J. C. WITH 10.0 ML. OF PLASMA B

The sedimentation rate was increased (maximum, 25 mm. per hour) from the 19th to the 36th days.

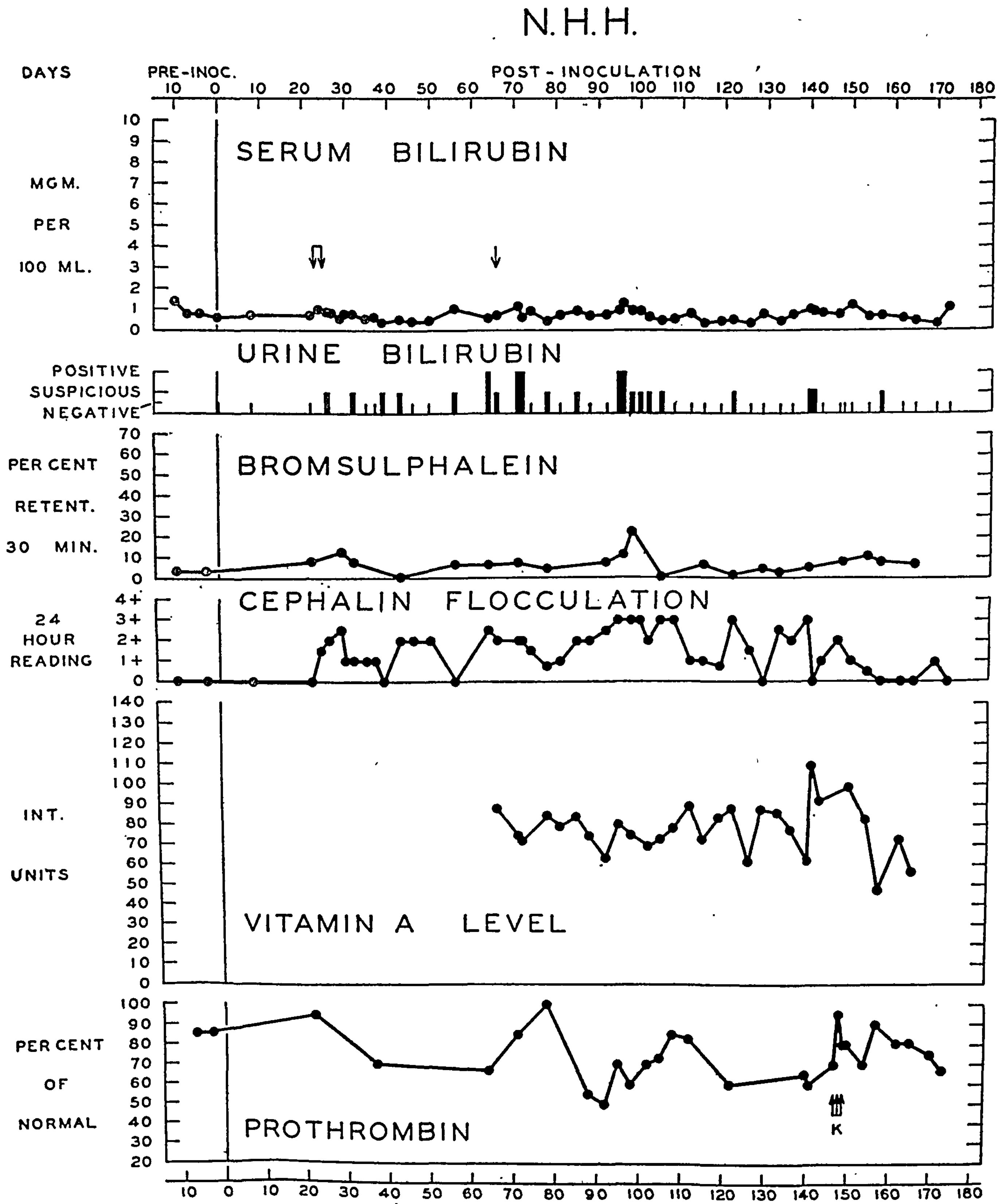

FIG. 7. STUDIES BEFORE AND AFTER THE INTRAVENOUS INOCULATION OF N. H. H. WITH 20.0 ML. OF PLASMA B

The sedimentation rate was increased (maximum, 28 mm. per hour) from the 29th to the 39th days. Arrows at K below the prothrombin curve indicate days when 5.0 mgm. of synkavite were given intravenously.

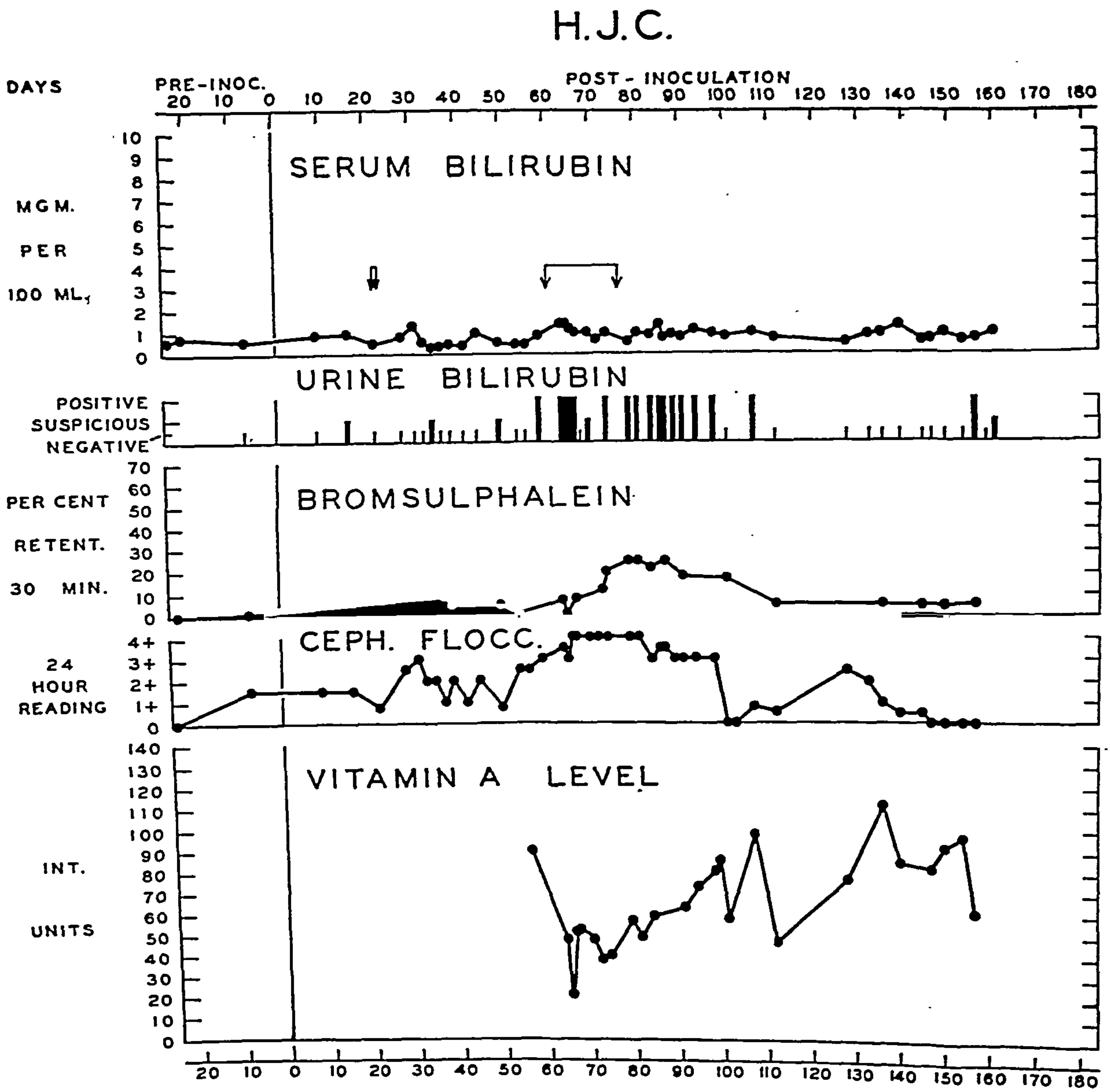

Fig. 8. Studies Before and After the Subcutaneous Inoculation of H. J. C. with 2.0 ml. of Yellow Fever Vaccine

5 days, and the subsequent interval, prior to the development of the final stage of the disease, was either asymptomatic or marked by periodic recurrences of mild symptoms and signs. The occurrence of abnormal liver function tests during this period indicated that the symptoms and signs were due, at least in part, to hepatic disturbance. During the *late period*, the symptoms and signs were more severe and prolonged, and they did not differ from those described by others as occurring in the prodromal phase of serum jaundice (1, 2, 4, 33). They included anorexia (8 cases), nausea (8), weakness and fatigue (8), dark urine (6), abdominal discomfort or pain (6), vomiting (4), upper respiratory infection (4), sensation of fever (4), skin eruptions (3), headache (3), eructation (3), arthralgia or joint stiffness (2), myalgia (2), sore throat (2), chilliness (1), and diarrhea (1). The physical findings during this period were not unusual.

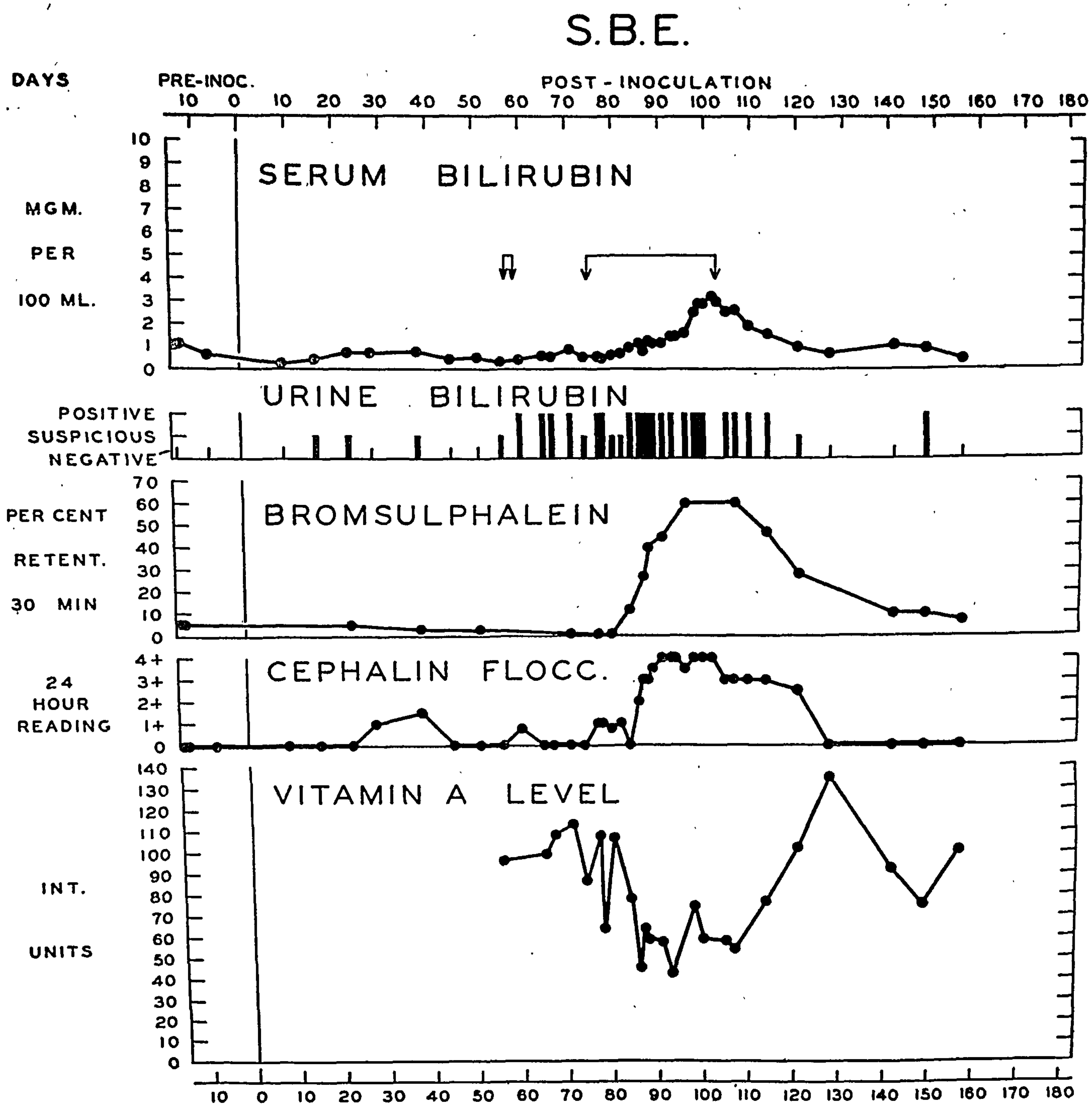

FIG. 9. STUDIES BEFORE AND AFTER THE SUBCUTANEOUS INOCULATION OF S. B. E. WITH 2.0 ML. OF YELLOW FEVER VACCINE

Overt jaundice was present in 5 cases. The liver was slightly enlarged and palpable for a brief period in most cases, but the spleen was palpable in only 1 (C. R. L.). The temperature was usually normal, although a few had occasional slight elevations. No hemorrhagic phenomena were observed. The cutaneous manifestations differed in the 3 cases. B. C. developed multiple pruritic lesions, similar to those of chronic eczema or urticaria, on both forearms, 40 days before the occurrence of overt jaundice, and these lesions persisted for 16 days. H. J. C. developed a macular erythematous rash over the ankles and lower legs 4 days before other evidence of acute hepatitis appeared. The lesions coalesced and the involved areas became fiery red. Swelling, local heat, and tenderness about the right ankle were associated. These phenom-

ena lasted for 9 days. F. S. developed severe generalized urticaria 4 days before other manifestations of hepatitis appeared, and these, together with associated swelling and pain in the joints of the knees, wrists, and hands, persisted for 6 days. Two of these 3 men had allergic histories and the other had had catarrhal jaundice 4 years before. The eruptions in all were suggestive of an allergic mechanism, and it seems possible that a sensitization to the agent or some one of its products may have resulted from its activity during the early period of the disease.

LABORATORY OBSERVATIONS

Most of the additional information concerning the course of this disease has been provided by the laboratory studies of hepatic function. Because of the minor and transient fluctuations in the results of the various tests in normal persons (Figure 10), the several preinoculation determinations have been of great value in the interpretation of the significance of small changes. The tests for serum bilirubin concentration, bilirubinuria, cephalin flocculation, bromsulphalein excretion, plasma vitamin A, and prothrombin activity were found to detect hepatic disturbance more consistently and promptly than did the tests for urine urobilinogen, total serum protein, albumin and globulin concentrations, total and esterified cholesterol, serum alkaline phosphatase, hippuric acid excretion and sedimentation rate. The results of all these tests have been included in Figure 1 because the case history is presented in this report, and also because this subject was the source of Plasma B. Figures 2 to 9, however, include only those tests which provided the most pertinent information. Figure 10 shows the results of the studies on a normal individual over a period of 60 days.

It will be noted that *bilirubinuria* was among the first signs of hepatic disturbance (Figures 2, 3, 6, 9). Moreover, it sometimes appeared long before there was any significant increase in the

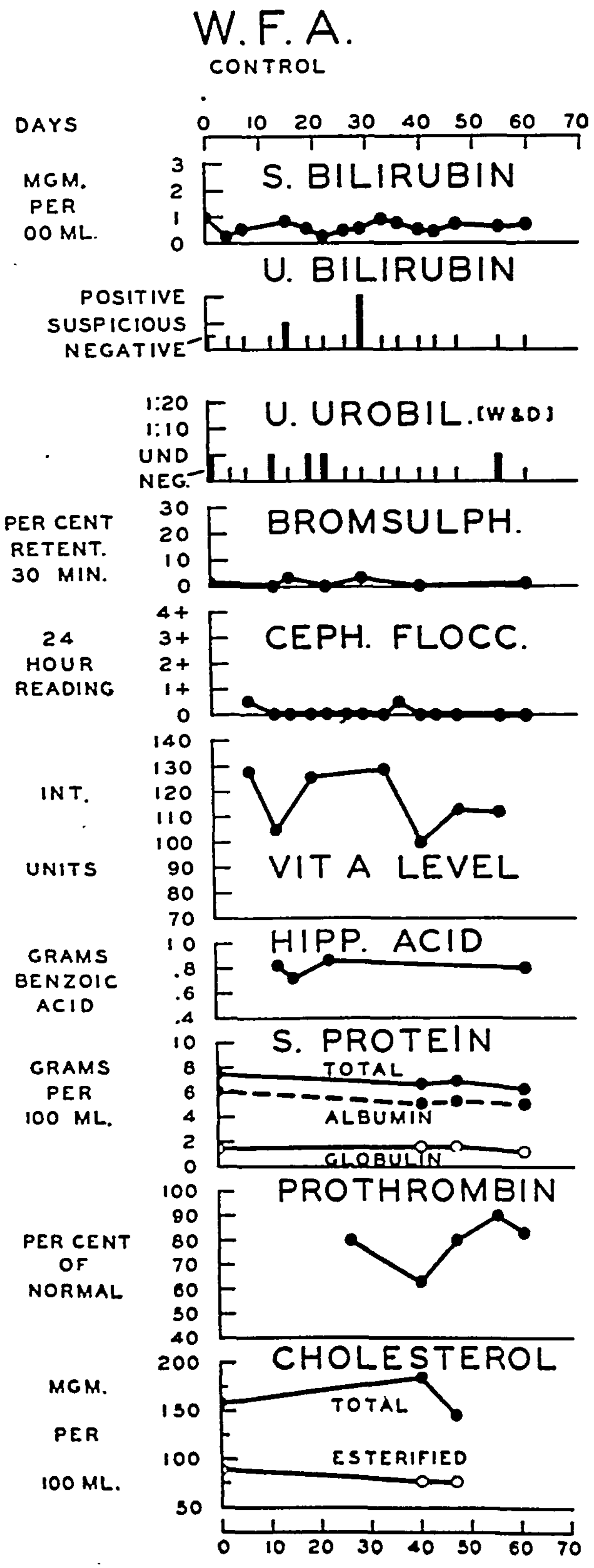

FIG. 10. STUDIES ON AN UNINOCULATED CONTROL FOR A PERIOD OF 60 DAYS

This individual worked and lived with the inoculated group. The only abnormal finding was the single positive urine bilirubin test. The fluctuations in vitamin A and prothrombin were within normal limits.

concentration of total serum bilirubin (Figures 2, 3, 9). In 1 case (Figure 9), bilirubinuria was the only definite finding that suggested activity of the disease during the symptom-free interval that immediately preceded the final stage of hepatitis with jaundice. The main disadvantage was the occasional finding of weakly positive tests in normals. The concentration of *serum bilirubin* showed transient slight elevations during the *early period* of the disease in some cases (Figures 1, 2, 3, 6, 8), but in others, no elevation was observed, although other tests revealed the presence of hepatic dysfunction during this period (Figures 7, 9). In those cases that developed overt jaundice during the late period, the serum bilirubin occasionally did not increase until a week or more after considerable hepatic dysfunction had been demonstrated by other means (Figures 2, 3). In 3 cases (Figures 6, 7, 8), the concentration of serum bilirubin never exceeded the upper extreme of the normal range for the group, although other tests showed considerable abnormality of hepatic function. This test, therefore, was useful, but a normal result did not exclude the presence of hepatitis. *The cephalin-cholesterol flocculation test* was helpful in spite of the limitations which have been previously discussed. Positive results were obtained in all cases during some stage of the disease, although in W. D. S. (Figure 5) the significance was questionable. In 5 cases (Figures 1, 6, 7, 8, 9), it suggested continued activity of the process during the asymptomatic intervals that followed the onset of the disease. In 2 instances during the late period of the disease, however, other procedures revealed hepatic disturbance 1 to 3 days before it was indicated by a positive cephalin test (Figures 3, 4). In only 1 case (Figure 1) was it the last test to return to normal after the final stage of the disease, negative results being obtained in other cases in which continued hepatic dysfunction was still revealed by bromsulphalein retention and bilirubinuria (Figures 2, 3, 4, 9). This is not surprising in view of the fact that the cephalin test is supposed to indicate only an active process. *The bromsulphalein test* proved to be a reliable and sensitive indicator of hepatic dysfunction, and in addition, it did not seem to be as subject to random influences as were many of the other procedures. Abnormal retention was observed in 4 cases during the mild transient episodes of hepatic disturbance that occurred in the early period of the disease (Figures 1, 6, 7, 8), and retention was always found during the more severe stages. The test was among the first to indicate the onset of the late stage of the disease (Figures 2, 3, 8, 9), and was usually one of the last to return to normal (Figures 2, 3, 4, 8, 9), thus affording one of the best indices of recovery. It was especially valuable in the cases of hepatitis without jaundice (Figures 6, 7, 8), and during the preicteric stage of cases which later developed jaundice. As jaundice was not severe in any of our cases, it was also used to advantage during the icteric stage. One man, B. C., developed a sensitivity to bromsulphalein after 7 injections, over a period of 4 months. This was first manifested by the occurrence of urticaria, 30 minutes after the injection. After the next injection, 1 week later, giant urticaria appeared within 5 minutes, and for this reason, the test was discontinued on this individual. *Plasma vitamin A determinations* were not available throughout the entire period of this investigation, but the results thus far suggest that the procedure is a sensitive indicator of hepatic disturbance. Decreases in the vitamin A level were observed simultaneously with, and occasionally before, changes in the results of other tests (Figures 2, 3, 6, 8, 9). It has therefore been of considerable value in a study such as this in which the type of disease is known in advance. However, its fluctuation in many varied conditions (35) greatly diminishes its value as a diagnostic test for hepatic disturbance in undiagnosed illnesses. *The determination of prothrombin activity* was helpful in some cases (Figures 2, 5, 6, 7), but in one (S. B. E.), it showed no significant change during a period of marked hepatic dysfunction with jaundice. The other studies previously mentioned were of value only in a confirmatory or negative way. In a few instances, the tests for hippuric acid excretion (Figure 1), urine urobilinogen (W. D. S.; B. C., Figure 1), and sedimentation rate (N. H. H., J. C.) added support to the evidence of hepatic dysfunction when the significance of changes in the results of other procedures was uncertain.

It is apparent that no single test provides reliable information concerning the hepatic func-

tional state at all times. A group of tests is therefore necessary, if mild hepatic disturbance is to be detected, and for this type of liver disease, the tests for serum bilirubin concentration, bilirubinuria, cephalin flocculation, bromsulphalein retention, plasma vitamin A level, and prothrombin activity appear to be the most satisfactory.

The *studies on the control group* indicated that the tests for serum bilirubin, urine bilirubin, cephalin flocculation, plasma vitamin A, cholesterol esters, prothrombin activity, and sedimentation rate occasionally yielded abnormal results in normal persons (Figure 10) or during incidental minor illnesses, unrelated to hepatitis. However, such abnormalities were never found with *more than one* of these tests at the same time. One person had repeated studies during a 10-day gastrointestinal upset in which the symptoms were similar to those observed in the volunteer group in association with mild hepatitis. In this case, all the tests were repeatedly negative. On the basis of these observations, it has seemed justifiable to regard abnormal results with more than one liver function test as evidence of hepatic disturbance, presumably due, in these cases, to hepatitis.

CASE REPORT

A brief summary of the case of B. C. has been included to illustrate the varied phenomena that may be associated with this disease.

B. C. (Figure 1), age 22, after the control studies, was inoculated intravenously with 1.0 ml. of Plasma A. *Twenty-two days* after inoculation he developed generalized aches and pains, headache, marked anorexia, nausea, vomiting, weakness, malaise, and elevation of temperature to 102° F. Examination revealed tenderness to fist percussion over the liver and a palpable spleen. Laboratory studies revealed an elevated serum bilirubin, retention of bromsulphalein, and increased urobilinogen in the urine. A number of "target cells" were observed in the blood smear. Plasma B was obtained from him on the 23rd day. Prompt recovery ensued and he was well until the *55th day* when anorexia returned, and was associated with vomiting and loose stools. The cephalin cholesterol test was weakly positive (1+). From the *60th to 76th day*, the eruption described previously appeared on the forearms. From the *72nd to 80th day*, malaise, anorexia, and sore throat were noted. The serum bilirubin rose on the *77th day* (Figure 1), but promptly returned to normal.

On the *85th day*, nausea, slight diarrhea, and vague persistent discomfort in the right lower abdomen occurred in association with focal tenderness in the right lower quadrant. The leukocyte count was 10,400 and temperature was 99.4° F. The surgeon advised laparotomy because of possible appendicitis. At operation, the appendix was normal, but the lower ileum was diffusely inflamed and the mesentery edematous and studded with swollen, enlarged lymph nodes. Post-operative roentgen examination of the gastrointestinal tract revealed no abnormality.

On the *109th day*, malaise, anorexia, nausea, and dark urine appeared, and were followed, on the *110th day*, by jaundice. On the *114th day*, he developed urticaria after injection of bromsulphalein. While still jaundiced, *118 to 123 days* after inoculation, there was slight elevation of temperature and a moderately severe diarrhea. On the *126th day*, roentgen examination of the gastrointestinal tract revealed an abnormal ileal pattern similar to that of regional ileitis, but the symptoms, by this time, had disappeared.

He was well after his discharge from the hospital on the *129th day*, and roentgen examination of the gastrointestinal tract on the *213th day* showed marked improvement of the small intestinal pattern, although slight abnormality still persisted. On the *232nd day*, he was in good health, but the cephalin test was still intermittently positive.

This case illustrates the occurrence of repeated manifestations of this disease before the development of jaundice. The demonstration of an acute ileitis and mesenteric adenitis at laparotomy is interesting in view of the post-mortem findings in the fatal cases of post-vaccinal hepatitis that occurred in the U. S. Army (1942). In these cases, acute inflammation and edema of various parts of the gastrointestinal tract were frequently observed (27). Symptoms of mild diarrhea and crampy abdominal pain, which in retrospect were probably due to this process, were experienced by B. C. intermittently throughout the pre-icteric and icteric periods. It seems likely that these symptoms and findings represent manifestations of a generalized disease in which only hepatitis is usually recognized. Apparently such findings have not previously been recorded as a manifestation of this disease except in autopsy material. Plasma B, obtained from this individual on the 23rd day, has been shown to contain the "hepatitis-producing" agent by the occurrence of a mild form of the disease in J. C. and N. H. H. following its injection.

POSSIBLE CONTACT CASES

Two hospital employees, who were housed separately from the volunteers and from each other, but who were in exceptionally close contact with one of the volunteers at a time when he was showing evidence of mild hepatitis, developed moderately severe jaundice within 1 to 3 months. The illnesses were typical of infective hepatitis (catarrhal jaundice). Both had moderate elevations of temperature (102° F.) during the prodromal stage, and in this respect, their disease differed from that of the volunteers, in whom only slight elevations were observed. The absence of other contact cases among the large institutional group, and especially among the 87 men with whom the volunteers were quartered, indicated that there was little tendency for the experimentally produced disease to spread by

ordinary contact. Because of the exposure of these 2 persons to the experimentally produced disease, and the absence of recognized exposure to other jaundiced patients or to each other, infection acquired from the volunteer remains a possibility. Propert (36) has previously recorded 2 cases of jaundice in which the infection appeared about 2 months after exposure to patients with measles serum hepatitis.

DISCUSSION

The fact that homologous blood products may contain an agent capable of producing hepatitis has been confirmed by the results of the inoculation of this group of men with plasma or yellow fever vaccine containing human serum. The high incidence (100 per cent) in this series is of interest in comparison with that reported by other investigators. Propert (36) recorded the occurrence of hepatitis in all of 7 children, each of whom received 4.5 ml. of one batch of convalescent measles serum, although a certain number of people elsewhere, receiving portions of the same batch of serum, did not develop hepatitis. Cameron (30) reported hepatitis in 86 per cent of 7 men inoculated with 1.0 to 2.0 ml. of blood or serum from patients with infective hepatitis. The onset of the disease in some of his cases was delayed as long as 6 months, and as the work was done in an endemic area, he points out that the experiment was not performed under ideal conditions. Oliphant, Gilliam, and Larson (2), working with a much larger group, recorded an incidence of only 20 to 30 per cent among those experimentally inoculated. It is apparent that the high incidences of successful experimental transmission have been confined to smaller series. In addition to the size of the groups, however, one or more of the following factors may be related to the higher incidence in the present series as compared with that of Oliphant *et al.*: (*a*) All of the volunteers here reported were between the ages of 19 and 25, whereas Oliphant *et al.* used subjects from 15 to 57 years of age. (*b*) The amount of material used for inoculation in our group was 1 to 100 ml. of plasma and 2 ml. of yellow fever vaccine. The dosage used by Oliphant *et al.* was much smaller, 0.1 to 0.16 ml. of serum and 0.5 ml. of vaccine. (*c*) The materials used in the present series may have contained a more virulent agent, although the mildness of the disease is somewhat against this possibility. (*d*) Mild transient cases, especially those without hyperbilirubinemia and demonstrable only by repeated liver function studies, may easily be overlooked. Also, in connection with the significance of the high incidence in the present group, it seems advisable to emphasize the apparent absence of other cases of jaundice among the large instutional group, with the exception of 2 cases that appeared after the disease had developed in the experimental group.

By the use of liver function tests, it has been ascertained that the onset of hepatitis, produced by an agent in serum, may be much earlier than has been generally recognized, a fact which may necessitate a revision of the current estimates of the incubation period of this disease. In 7 of the 9 volunteers, the incubation period, measured from the time of inoculation to the first manifestations of hepatitis, was 12 to 50 days, and in 6 of these, it was between 12 and 35 days. It thus appears that overt jaundice is probably a late manifestation of this malady, and one which, as indicated by 3 of the present cases, may not appear at all. The shorter incubation period directly concerns the relation of this disease to infective hepatitis. The possible etiologic identity of these diseases has been discussed repeatedly (33, 37, 38) but no conclusions have yet been reached. In this regard, the 2 alleged forms of infective hepatitis must be briefly mentioned. (*a*) Single sporadic cases occurring without apparent contact with other cases of jaundice are frequently seen, and they, in turn, often do not seem to infect others with whom they are in close contact. This type of the disease apparently has little tendency to spread. However, this or a similar disease occasionally occurs in, (*b*) a distinctly epidemic form. It is not yet known whether these 2 types are caused by the same or different agents. The same problem exists in connection with the relationship of the causative agent or agents of these types of hepatitis to that which is transmitted by serum. An apparently shorter incubation period, the more frequent occurrence of fever, and the contagious nature have been cited as features of the epidemic type of hepatitis that suggest a difference between its causative agent and that of serum jaundice (2, 37, 38). It

has been indicated by the present study that the periods of incubation may coincide. The occurrence of fever is a questionable means of distinction, for it may be present in some cases transmitted by serum and absent in some cases of infective hepatitis unrelated to serum. Furthermore, minor differences in the clinical manifestations of serum hepatitis might exist because of (*a*) the differences in the portal of entry of the agent (37, 38), (*b*) the conditions under which the agent or agents have existed prior to entry, such as desiccation, and (*c*) the possible presence of immune bodies in "hepatitis-producing" serum or plasma pools that might be temporarily protective. Thus, the length of the incubation period, the clinical phenomena, and the pathologic changes (27, 28) do not appear to provide a satisfactory basis for distinguishing between the etiological agents of these diseases. The tendency of the epidemic type of hepatitis to spread among contacts, therefore, seems to be the chief remaining point of distinction. There is as yet no unequivocal evidence that serum hepatitis is contagious, the majority of reports indicating that the disease has little tendency to spread (2, 4). The transmission of the agent of serum hepatitis by nasopharyngeal washings (37) suggests that contact infection may be possible, but this work has not yet been confirmed. The 2 cases reported by Propert (36) and the 2 possible cases cited herein apparently represent the only recorded instances in which contact infection may have occurred under natural circumstances. The non-contagious nature of the type of serum hepatitis that occurred in this group, in that of Oliphant *et al.* (2), and in the U. S. Army (1942) (4), suggests that the disease may be caused by a different strain of the same agent or by a different agent than that which causes the epidemic type of infective hepatitis. This distinction does not apply, however, to the sporadic form of infective hepatitis, for there is as yet no conclusive evidence that supports a differentiation of its causative agent from that of serum hepatitis.

Hepatitis without jaundice. Eppinger (29) has described several cases in which severe liver damage was found at autopsy in patients who had had no hyperbilirubinemia. For these cases, he used the term hepatitis *sine ictero*. Such terminal observations are quite different from the detection of hepatitis *sine ictero* in living subjects. Van Rooyen and Gordon (32) suspected the occurrence of hepatitis without jaundice, and Cameron (30) has stated: "other evidence from Palestine seems to show that jaundice is not essential and that a subdivision into hepatitis *cum ictero* and *sine ictero* may be justified."[12] The evidence from Palestine was not given, perhaps due to wartime restrictions, so that the data presented herein apparently provide the best evidence, to date, of hepatitis *sine ictero* in living subjects. As cases of this type have unquestionably been overlooked, it is probable that the available statistics afford an erroneous estimate of the true incidence of hepatitis. In addition, the recognition of hepatitis without jaundice will perhaps lead to a better understanding of the course and manifestations of the disease.

Summarizing the observations in this group, the stages of serum hepatitis may be tentatively outlined as follows:

$$\text{Inoculation} \xrightarrow[\text{days}]{\text{12 to 35}} \begin{matrix}\text{Mild}\\ \text{hepatitis}\\ \text{without}\\ \text{jaundice}\end{matrix} \xrightarrow[\text{days}]{\text{65 to 110}} \begin{matrix}\text{Hepatitis}\\ \text{with or}\\ \text{without}\\ \text{jaundice}\end{matrix}$$

Variations from this simple outline have been noted, and its revision may be necessary when more cases have been studied. The results of this investigation provide no direct evidence concerning the etiological agent(s) involved. Nevertheless, the demonstration of an early, nonicteric stage, and the finding that plasma taken during this stage contained the "hepatitis-producing" agent are facts which may lead to a better understanding of the epidemiology of serum hepatitis.

SUMMARY

The fact that plasma or serum may contain a transmissible agent, that is capable of producing hepatitis, has been confirmed by the results of the inoculation of 9 human volunteers. Of the

[12] Following the submission of this manuscript for publication, a report by MacCallum and Bauer appeared in the Lancet (1944, 1, 622). The occurrence, in a few inoculated volunteers, of minor symptoms in association with slight elevations of serum bilirubin insufficient to cause overt jaundice, also led them to the concept of hepatitis without jaundice.

5 subjects who received intravenous injections of a pooled mumps convalescent plasma, 4 developed hepatitis with jaundice, and the 5th showed evidence of mild hepatitis without jaundice. Two men received intravenous injections of plasma that was obtained from one of the above group 23 days after his inoculation; both of them developed mild hepatitis without jaundice. The donor of this plasma developed jaundice 87 days after the plasma had been taken. Two men given subcutaneous inoculations of yellow fever vaccine developed hepatitis, one with and one without, jaundice. The incidence of hepatitis in the total group, therefore, was 100 per cent. Several of these cases would not have been detected if liver function studies had not been carried out at frequent intervals. One volunteer, who was reinoculated with the same material after recovery from the first attack, has shown no evidence of hepatitis during the 140 days that have thus far elapsed since the second inoculation.

In 6 of the 9 cases, the onset of the disease occurred from 12 to 35 days after inoculation. The contrast of this observation with previous estimates of the incubation period of this disease is discussed. Experiences with a rather large group of liver function studies are presented and their value in the study of this disease is emphasized. The advantages and limitations of certain of the individual tests are considered. Laboratory data demonstrating the occurrence of hepatitis without jaundice are presented. Observations suggesting the generalized nature of this disease are found in the report of a case in which ileitis and mesenteric adenitis were seen at laparotomy. Two cases of jaundice that may have occurred as a result of contact with one of the volunteers are briefly described. The possible relation of "serum hepatitis" to the various forms of infective hepatitis is discussed.

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TABLE II

Comparison between the plasma clearances of urea and of thiourea

Calculated on the basis of urea and thiourea concentrations in serum water

Case	Urine volume	Plasma clearance		Ratio, $C_{urea}/C_{thiourea}$
		Urea	Thiourea	
	ml. per min.	*ml. per min.*	*ml. per min.*	
1 fasting	1.46	54.2	55.7	0.97
	1.97	87.3	85.6	1.02
	1.41	66.1	64.1	1.03
	1.74	60.2	60.8	0.99
1a not fasting	2.13	59.2	57.2	1.03
	1.19	49.7	50.6	0.98
	1.27	58.2	56.9	1.02
	1.38	59.8	50.9	1.17
	3.11	68.8	56.7	1.21
	1.68	47.6	47.4	1.00
Average				1.04

The last column in Table I gives the average concentrations of thiourea in serum water at different times after the ingestion of the thiourea. The descending curve for these averages is not so smooth as for individual cases, because the averages at some periods are for only 1 or 2 subjects. In general, the serum concentration has fallen to half its maximum at about the tenth hour.

DISCUSSION

As for the measurement of total body water, there is not much to be said except that thiourea is not suitable for this purpose, as was found in dogs. The promising results, mentioned at the start of this paper (3), might possibly depend upon the inability of the body to metabolize or store more than a few hundred mgm. of thiourea during the period of the test. Such losses from the several thousand mgm. doses which they used would give only small percentage errors.

As for the disappearance of thiourea in the body, it is reasonable to conjecture that the antithyroid effect of this substance depends upon the retained (unrecovered) fraction. Since the concentration in serum falls rather rapidly, perhaps the use of divided doses during the day would prove more effective in inhibiting thyroid activity than would a single daily dose. One thinks of sulfonamide therapy, in which a primary dose is given to obtain the required blood level, which is then maintained by giving smaller divided doses at suitable intervals.

CONCLUSIONS

Thiourea is not suitable for measuring total body water.

When thiourea doses of 1000 mgm. are given by mouth, about 25 per cent of the ingested thiourea cannot be recovered.

None of the ingested thiourea appears in the feces obtained over a 3-day period afterward.

The concentration of thiourea in breast milk approaches very closely the concentration in serum.

Thiourea enters the cerebrospinal fluid, probably slowly.

The renal clearance of thiourea is very close to that of urea.

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CHEMICAL STUDIES IN HYPERTENSION. REDUCING AND NITROGENOUS FRACTIONS IN PROTEIN-FREE BLOOD FILTRATES

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The literature concerning non-glucose reducing substances in the blood in clinical states involving vascular hypertension has apparently been limited to observations incidental to other problems. In 1925, certain investigators (1) found some increase in the non-glucose reducing fraction of zinc hydroxide (Hagedorn-Jensen) blood filtrates from patients with glomerulonephritis, some of whom presumably had hypertension. In 1927, Somogyi (2) noted an increase of reducing non-sugars in tungstic acid filtrates from patients with high nitrogen retention. Other authors (3), in 1929, reported significant amounts of non-fermentable reducing substance in mercury filtrates from 2 patients with advanced hypertensive disease.

This report describes primarily the determination of non-fermentable reducing substance and non-urea nitrogen in protein-free blood filtrates from hypertensive subjects. The patients are classified into 2 groups, one with normal, the other with elevated blood non-protein nitrogen levels, in an effort to demonstrate any corresponding variations in the fractions under consideration.

EXPERIMENTAL PROCEDURE

Blood for analysis was drawn into flasks containing sodium oxalate as anticoagulant. Deproteinization and analysis were performed promptly. Some filtrates were stored overnight in stoppered bottles, in a cold room at 3 to 4° C., pending analysis. This did not appear to affect the results in any way. Most of the blood samples were obtained under fasting conditions. Urea clearances were determined by a method described in 1928 (4). Mean blood pressures were recorded.

ANALYTICAL METHODS

Zinc hydroxide precipitation was performed according to Somogyi (2). Tungstic acid precipitation was carried out by the Folin and Wu (5) technic, as modified by Haden (6). These methods were selected because of their convenience for the study of reducing and nitrogenous fractions. Tungstic acid filtrates contain apparently maximum amounts of these fractions, which are considerably restricted in zinc filtrates, by removal of glutathione, ergothionine, uric acid, etc. Non-protein nitrogen was determined by the method of Folin and Wu (5), by use of superoxol to clear the digestion mixtures when necessary. Urea nitrogen was estimated by the gasometric hypobromite method of Van Slyke and Kugel (7). Total reducing substance was determined with the copper-iodometric reagent of Shaffer and Somogyi (8), containing 5 grams of potassium iodide per liter. For non-fermentable reducing substance, a reagent containing 1 gram of potassium iodide per liter was employed. Glucose was removed with washed yeast (Fleischmann's starch-free baker's yeast) by the method of Somogyi (2). All analyses were made in duplicate.

Values for non-fermentable reducing substance have been given in terms of glucose-equivalents, and therefore have only relative significance. The limits of error in the analytical method employed for estimating this fraction were found to be less than 1 mgm. of glucose per 100 cc.

RESULTS

Table I presents the results on analysis of zinc filtrates from the control subjects.* The ranges of non-protein nitrogen and urea are within the limits given by Somogyi (2) and by Peters and Van Slyke (9). The analytical results for non-fermentable reducing substance are in fair agreement with those given by Somogyi (2), there being no appreciable amount in 17 out of 21 filtrates.

Table II contains the findings on analysis of zinc filtrates from a group of hypertensive subjects with blood non-protein nitrogen below 30 mgm. per 100 cc. The range of the nitrogen fractions is very close to that in Table I. Appreciable non-fermentable reducing substance is present in 20 out of 27 filtrates. In 13 instances, the amount is greater than that found in any control filtrate.

Table III gives analytical data on zinc filtrates from a group of hypertensive subjects with blood

TABLE I

Observations on zinc filtrates from control subjects

Subject	Age	Diagnosis	Blood pressure	Urea clearance	N P N	Urea N	Non-urea N P N	Reducing substances, estimated as glucose: Total	Reducing substances, estimated as glucose: Non-fermentable
	years			*per cent of normal*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
1	21	Acute lead poisoning, latent syphilis, secondary anemia	124/80		24	10	14	92	0
2	35	Diabetes mellitus, 40 units insulin daily	115/80	95	22	13	9	84	0
3	18	Subacute appendicitis	112/64	118	19	9	10	68	0
4	26	Rheumatic heart disease	140/20	73	33	19	14	75	0
5	40	Bronchial asthma	124/100	127	25	17	8	58	1
6	50	Diabetes mellitus	126/86	81	21	13	8	222	0
7	34	Acute salpingitis	118/70		18	7	11	62	0
8	19	Gigantism, traumatic ulcer	140/80	108	20	11	9	86	2
9	24	Healthy	108/64	121	17	8	9	74	0
10	17	Allergic eczema	124/88	109	19	12	7	50	0
11	27	Healthy	112/70	88	21	10	11	78	0
12	18	Peptic ulcer	122/82	111	19	10	9	68	0
13	18	Sacro-iliac strain	120/75	132	23	12	11	75	1
14	41	Chronic sinusitis, chronic cholecystitis	124/80		26	17	9	60	0
15	59	Allergic dermatitis	114/72	82	19	8	11	73	0
16	37	Healthy	125/75	83	22	11	11	78	0
17	23	Bronchial asthma	95/70	99	20	7	13	45	0
18	23	Healthy	112/70	116	20	12	8	88	0
19	24	Acute tonsillitis, rheumatic arthritis	130/86		23	11	12	98	0
20	22	Healthy	138/84	95	18	8	10	82	0
21	27	Gonorrheal arthritis	150/88	110	23	12	11	64	1
Mean	29		123/75	103	22	11	10	80	0.2
Standard deviation				±16.6	±3.6	±3.2	±1.9	±34.3	±0.5

non-protein nitrogen of 30 mgm. per 100 cc. and over. Corresponding to expectation, there is elevation of the non-urea nitrogen as compared with Tables I and II. Demonstrable amounts of non-fermentable reducing substance are present in 10 out of 11 filtrates, with a mean value more than double that in Table II. The difference between these means appears significant, since its probable error is ± 0.5 mgm. per 100 cc., or less than one-fourth of the difference.

There does not appear to be any correlation between the amounts of non-fermentable reducing substances shown in Tables II and III with blood pressures. The relation to urea clearances is uncertain. In Table III, the two highest values for non-fermentable reducing substance occur in subjects with clearances below 10 per cent of normal. With higher clearances, there does not appear to be any correlation. There is some tendency for non-urea nitrogen to vary with non-fermentable reducing substance, the correlation coefficients in Tables II and III being +0.26 and +0.30, respectively.

Classification of the subjects in Tables II and III according to clinical diagnoses shows that appreciable amounts of non-fermentable reducing substance were present in each of 6 cases of malignant hypertension, with a mean value of 3.3 mgm. per 100 cc. (referred to glucose); in 15 out of 21 cases of benign hypertension, with a mean value of 1.9 mgm. per 100 cc.; and in 4 out of 5 cases of arteriosclerosis with hypertension, with a mean value of 2.0 mgm. per 100 cc.

Tungstic acid filtrates from groups of control and hypertensive subjects without nitrogen retention were analyzed in a similar way. The mean values for non-protein nitrogen in the two groups were almost identical: 24.9 mgm. per 100 cc. for the controls, 24.8 mgm. per 100 cc. for the hypertensives. The mean value for non-fermentable reducing substance in the control group was 20.9 mgm. per 100 cc., in close agreement with

the figure of 21 mgm. per 100 cc. given by Somogyi (2). A mean value of 22.3 mgm. per 100 cc. was obtained in the hypertensive group. The variation within the groups was such as to tend to obscure the significance of any difference between the means.

A few preliminary experiments were made relative to the non-fermentable reducing substance present in zinc filtrates from subjects with hypertension. It appeared to be stable on storage in the cold and to reside chiefly in the plasma. Attempts at acid hydrolysis of filtrates resulted in little or no change in the amount of non-fermentable reducing substance.

DISCUSSION

As suggested by Hiller, Linder, and Van Slyke (1), it is entirely possible that retained nitrogenous substances, such as uric acid and creatinine, may account for abnormal reducing properties in appropriate blood filtrates. Somogyi (2) found that zinc filtrates of blood from normal subjects prepared by his method contain somewhat less creatinine than tungstic acid filtrates and are free from uric acid, glutathione, and ergothionine. The nature of the non-urea nitrogen and non-fermentable reducing substance in these filtrates remains largely unknown. Retention of crea-

TABLE II

Observations on zinc filtrates from hypertensive subjects with non-protein nitrogen below 30 mgm. per 100 cc.

Subject	Age	Diagnosis	Blood pressure	Urea clearance	N P N	Urea N	Non-urea N P N	Reducing substances, estimated as glucose	
								Total	Non-fermentable
	years			*per cent of normal*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
22	33	Essential hypertension, hyp. ht. dis., chr. bronchitis	190/110	80	21	8	13	80	5
23	44	Essential hypertension, hyp. ht. dis.	155/105	85	26	17	9	84	1
24	50	Essential hypertension, hyp. ht. dis.	268/140	79	18	10	8	178	0
25	54	Essential hypertension, hyp. encephalopathy	190/108	103	22	12	10	79	4
26	47	Essential hypertension, hyp. ht. dis.	178/98	79	24	12	12	78	0
27	21	Malignant hypertension	220/118	84	21	12	9	148	2
28	54	Essential hypertension	168/98	92	20	12	8	143	1
29	37	Essential hypertension, hyp. ht. dis., thyroidectomy	218/110	77	21	11	10	54	1
30	52	Essential hypertension	190/130	126	26	14	12	99	2
31	33	Malignant hypertension, lt. lumbar sympathectomy	174/110	97	17	8	9	87	4
32	39	Essential hypertension, hyp. ht. dis.	220/100		17	10	7	150	1
33	54	Arteriosclerosis with hypertension	264/130	96	20	8	12	43	3
34	66	Essential hypertension, hyp. ht. dis.	185/90	171	17	8	9	82	3
35	35	Malignant hypertension, CNS syphilis	220/130	90	23	14	9	106	1
36	46	Arteriosclerosis with hypertension	176/110	80	25	13	12	62	1
37	35	Essential hypertension, hyp. ht. dis.	176/100	85	18	7	11	89	0
38	49	Essential hypertension, diabetes, obesity, goiter	204/114	87	19	9	10	57	0
39	53	Essential hypertension	210/115	157	19	8	11	132	3
40	39	Essential hypertension	196/128	83	19	10	9	94	0
41	37	Thyrotoxicosis, thyrotoxic ht. dis.	181/85	133	20	8	12	72	3
42	28	Malignant hypertension, hyp. encephalopathy	180/130	55	29	18	11	63	3
43	58	Essential hypertension, hyp. ht. dis.	214/126	68	17	9	8	90	1
44	68	Arteriosclerosis with hypertension, hyp. ht. dis.	200/110	68	22	13	9	87	0
45	66	Essential hypertension, hyp. ht. dis.	185/90	59	20	10	10	64	3
46	56	Hyperthyroidism	200/104	45	24	16	8	112	2
47	62	Essential hypertension, hyp. ht. dis.	194/104	59	27	13	14	126	0
48	42	Essential hypertension	170/120	63	18	6	12	107	3
Mean	47		197/112	89	21	11	10	95	1.7
Standard deviation				±29.7	±3.4	±3.1	±1.8	±33.1	±1.5

TABLE III

Observations on zinc filtrates from hypertensive subjects with non-protein nitrogen 30 mgm. per 100 cc. and over

Subject	Age	Diagnosis	Blood pressure	Urea clearance	N P N	Urea N	Non-urea N P N	Reducing substances, estimated as glucose: Total	Reducing substances, estimated as glucose: Non-fermentable
	years			*per cent of normal*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
49	59	Arteriosclerosis with hypertension	190/100	78	36	24	12	78	3
50	45	Essential hypertension	190/125	19	61	42	19	68	3
51	40	Malignant hypertension	212/142	8	84	62	22	66	7
52	57	Malignant hypertension, hyp. ht. dis.	260/160	48	42	31	11	76	3
53	67	Essential hypertension, hyp. ht. dis., cor. art. dis.	218/110	43	36	21	15	100	4
54	62	Essential hypertension, cor. art. dis.	220/98	59	30	22	8	101	5
55	24	Chronic hemorrhagic nephritis	155/95	32	38	23	15	82	0
56	58	Arteriosclerotic nephritis	180/120	24	45	35	10	94	3
57	59	Arteriosclerosis with hypertension	220/148	15	85	72	13	164	3
58	28	Chronic hemorrhagic nephritis, uremia	220/115	4	135	118	17	73	8
59	29	Pituitary basophilism?	180/110	58	31	21	10	151	4
Mean	48		204/120	35	57	43	14	96	3.9
Standard deviation				±23.8	±32.6	±30.4	±4.3	±32.9	±2.2

tinine might account for the larger amounts found in Table III, as compared with Table II. A slight association between these fractions is suggested by analysis of the data. The correlation is of a low order, however, and the tendency for non-fermentable reducing substance to appear before the non-protein nitrogen rises suggests that the former may contain little or no nitrogen under the conditions imposed. This would be in agreement with the observations of West, Scharles, and Peterson (3) on mercury filtrates, which were found to contain increased amounts of non-fermentable reducing substance in 2 cases of hypertensive disease, but were generally free from nitrogen.

It may be significant that the two highest values for non-fermentable reducing substance in zinc filtrates occurred in those from patients with urea clearances less than 10 per cent of normal. If the limited number of subjects and the variation inherent in the urea clearance are taken into account, the apparent absence of progressive accumulation of this reducing fraction with moderate decrease in renal function suggests that it is either readily excreted or destroyed under such conditions. The increase with severe renal damage might be interpreted to mean that it is eliminated largely via the kidneys,—perhaps by a mechanism analogous to that for urea, which does not tend to accumulate in the body fluids until its renal clearance has been lowered to less than half its usual value. This would be in agreement with the observation by West (10) that appreciable amounts of non-fermentable reducing substances are present in normal urine. West pointed out that the amount eliminated is related to the carbohydrate intake, and particularly to the ingestion of caramelized material. Whether this has any bearing on the non-fermentable reducing substance noted in the present study is a matter for investigation.

Attention is called to the possibility that reducing substances might tend to accumulate in the blood or urine by interference with renal oxidations. Glaser, Laszlo, and Schürmeyer (11) have shown that mammalian kidneys normally require up to 20 per cent of the total oxygen uptake. These investigators, confirmed by Van Slyke, Rhoads, Hiller, and Alving (12), report a linear relationship between renal blood flow and oxygen consumption, both of which are unrelated to excretory activity. Studies of hypertensive subjects by indirect methods, summarized recently by Foà, Woods, Peet, and Foà (13), indicate a fairly general tendency toward lowering of renal blood flow in these subjects. It would seem to follow, *pari passu*, that there might exist a corresponding tendency toward impairment of

renal oxygen consumption. As a working hypothesis, it is suggested that renal ischemia, by interfering with intense cellular oxidations, may give origin to unusual amounts of incompletely oxidized substances. This hypothesis should be susceptible to direct experimental examination.

The preliminary examination of zinc filtrates containing non-fermentable reducing substance suggests that it is stable and exists chiefly in the plasma. This latter finding is in contrast with the studies of Somogyi (2) on tungstic acid filtrates, in which the bulk of the non-fermentable reducing fraction was shown to originate in the cells.

SUMMARY

1. Protein-free blood filtrates from subjects with and without arterial hypertension have been examined for non-fermentable reducing substance and non-urea nitrogen content.

2. Appreciable amounts of non-fermentable reducing substance were noted in 30 out of 38 zinc filtrates from patients with hypertensive disease, and in 4 out of 21 control filtrates. A similar difference was found in tungstic acid filtrates.

3. Among the hypertensive subjects, the non-fermentable reducing substance in zinc filtrates exhibited some tendency to vary with non-urea nitrogen. No direct relation to blood pressure or urea clearance could be demonstrated.

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VARIATION IN BEHAVIOR OF BUFFY COAT CULTURES AMONG INDIVIDUALS OF DIFFERENT CONSTITUTION TYPES[1]

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In view of well established gross constitutional similarities among subjects of a given disease, based on morphology and personality evaluation, the question arose whether analogous phenomena might likewise extend into the microscopic aspects of these subjects; whether the cells of all bodily tissues may show recognizable characteristics of appearance and reaction which stamp upon them an individuality comparable to that which marks the whole animal. The cells most readily available for such study and those which easily display observable qualities, because of their motility, are the leukocytes of the circulating blood. Consequently, an investigation of the buffy coat by tissue culture was undertaken.

Since the early cultures of human leukocytes by Awrorow and Timofejewskij in 1914 (1), the technic has been modified in various ways and has become a successful method for studying the behavior and developmental potentialities of the white blood cells. The chief concern of previous investigators has been with the sensitivity of fibroblasts and monocytes to their environment. Such studies have dealt with colonies of cells maintained *in vitro* for several days or weeks.

The present investigation is restricted to the first 24 to 30 hours of *in vitro* existence, during which time only slight changes in cell form have taken place. Freshly explanted cells have earlier been employed by Chambers and Grand (2, 3) in their studies of chemotactic reactions of leukocytes, and by Mallery and McCutcheon (4) who showed that the activity of freshly drawn leukocytes varies with the state of health of the patient from which they are taken. In the case of patients acutely ill with certain infectious diseases, cell motility is decreased. Lewis (5, 6) has described the locomotion of both neutrophilic leukocytes and lymphocytes in culture media. Finally, Carrel (7) has shown that buffy coat cultures from the blood of certain animal species take on individual patterns *in vitro* (5 to 6 days). Repeated cultures from the blood of a given individual yield identical cell patterns. The work of the above mentioned investigators has in many cases touched upon the problem of specific and even individual differences, but no comparative study, using man as the subject, appears to have been undertaken by this method.

Our object in this research was to investigate the possible existence of constitutional characters in the form and conduct of cells from the blood of human beings who were subjects of acute anterior poliomyelitis.[2] For controls, buffy coats from constitutionally stamped susceptibles to three other diseases, namely peptic ulcer, coronary occlusion, and gall bladder disease, were used. These particular groups were chosen because it had already been shown that they display the greatest gross constitutional differences. Preliminary observation demonstrated that the greatest contrast in cell culture characteristics obtains between cultures from subjects of poliomyelitis and of gall bladder disease. The other controls, those bearing peptic ulcer and coronary disease, were found to be disposed at intermediate points between these two extremes. Now if the hypothesis of constitutional susceptibility is correct, then the individual characteristics should be demonstrable at any time in the subject's life. However, the obvious temporary changes which the body undergoes in acute, especially febrile illness, may to some extent distort or obscure some of the permanent criteria of the individual's personal identity in health. For this reason, blood was taken from

[1] Aided by a grant from the National Foundation for Infantile Paralysis, Inc., N.Y.C.

[2] Poliomyelitis cases were made available through the courtesy of Willard Parker, Orthopedic, and Ruptured and Crippled Hospitals and the Jersey City Medical Center.

some patients in the acute phase of infantile paralysis and from others during later stages of recovery. Indeed some of them had had their acute illness five to thirty years before. The latter were chosen in order to secure blood whose numerical and differential white cell counts were normal in the ordinary sense. From these observations, we have gained the impression that the culture characters herein described do not represent changes caused by the presence of the disease itself, but like the gross factors also express an aspect of the individual's constitution.

METHOD

The general picture of leukocyte migration in a plasma clot medium, as has been repeatedly shown by previous investigators, is as follows: The cells from the explanted buffy coat, incubated at 37.5° C., begin to migrate outward in a uniform corona almost immediately, so that within 1 or 2 hours a macroscopically noticeable areola is formed. Monocytic cells preponderate in the *van*. *Neutrophil leukocytes* appear somewhat later and move outward more slowly. They are therefore found in greatest numbers near the explant. In 24 hours the extent of the areola is no longer well defined; it has become diffuse and generally covers the greater part of the area available. By this time the neutrophil leukocytes have begun to degenerate and disappear as have also some of the lymphocytes. Mononuclear cells have begun to increase in number by division. Changes in size and staining properties make it impossible to distinguish between lymphocytes and monocytes in most cases (8).

In the present studies, the following procedure was used:

A 7 cc. sample of blood was drawn into a syringe containing 1.1 cc. heparin in 1:2000 dilution. Further anticoagulant measures included maintenance of materials at low temperature and the use of tubes previously treated with NaOH for collection of blood, and tubes lined with paraffin for plasma.

Blood was centrifuged at 3500 r.p.m. for 15 minutes and the supernatant plasma pipetted off. The buffy coat was removed and divided into fragments about 1 mm. in diameter. Tissue culture preparations were made by the Maximov slide method; 12 explants were usually prepared from the buffy coat of each patient's blood. All operations were carried out under sterile precautions.

The preparations were incubated at 37.5° C. and observed at appropriate intervals. Since we were concerned principally with cells in their initial aspect rather than with transformation forms, cultures were maintained only 30 to 48 hours after explanation. The advance of cells into the culture medium was recorded by measuring the average width of the areola (to the nearest 0.1 mm.) at 3½ and 24 hours, respectively. Records were kept of the degree of activity evinced by the cells, and of the types of cells present; cells were typed according to form and motility, not by hematological groups. Whole cultures were fixed and stained with Delafield's hematoxylin and eosin or with toluidine blue and eosin during early hours of incubation and at approximately 24 hours of incubation. Differential counts of hematological cell types were made for the 24-hour cultures, fields being counted at all levels of the areola from explant to advancing edge, including approximately 1000 cells. Total numbers of cells present in the entire areola, type distribution, etc., were then calculated.

PROCEDURE

Three hundred and twenty-eight cases, selected solely on the basis of the disease from which they had suffered were studied. As the work progressed, it soon appeared that age and sex played a minimal rôle in the phenomenon of culture growth. For this reason, these two factors were disregarded in the calculations. Characteristics of areola formation, rate of increase in areola size, aspect of the advancing margin, and cell form are best defined in most cases during the period from 2 to 5 hours after explantation. Further information on activity, shapes, and disposition of the various cells can be obtained after 24 hours of incubation. The various observations were therefore made on all cultures at these two stages.

Cultures from subjects of the 4 different maladies have been found to vary in the following respects:

1. *Areolar size.* After 3½ hours' incubation the areola breadths were measured and the following results obtained (Table I):

TABLE I

Range, mean, and standard deviation of areola sizes after 3½ hours' incubation

	No. cases	Range	Mean and P.E.	S.D.
Poliomyelitis	28	0.4 to 1.3 mm.	0.79 ± 0.04	0.23
Peptic ulcer	35	0.5 to 1.5 mm.	0.86 ± 0.03	0.25
Coronary disease	20	0.9 to 1.8 mm.	1.19 ± 0.03	0.22
Gall bladder disease	48	0.5 to 1.7 mm.	1.19 ± 0.02	0.26

From the above table, it appears that the areolas at 3½ hours of poliomyelitis and ulcer patients average smaller than those from coronary disease and gall bladder disease. The averages for the former 2 groups are approximately 0.8 mm. and for the latter groups, 1.2 mm.

That the differences between mean values are

statistically significant and not due to sampling errors is indicated in Table II where the XPEs [8] of these compared groups are for the most part above the significant level of 3.

TABLE II

Differences of means and XPEs for intergroup comparisons of areola size, after 3½ hours' incubation

	Difference and PE	XPE
Poliomyelitis/Ulcer	0.07±0.05	1.40
Poliomyelitis/Coronary	0.40±0.05	8.00
Poliomyelitis/Gall bladder	0.40±0.045	8.89
Ulcer/Coronary	0.33±0.042	7.85
Ulcer/Gall bladder	0.33±0.036	9.17
Coronary/Gall bladder	0	0

The same relative degree of difference in areola size was also evident in the cultures after 24 hours of incubation. Table III sets forth the values obtained for the 4 disease groups.

TABLE III

Range, mean, and standard deviation of areola sizes after 24 hours' incubation

	No.	Range	Mean and P.E.	S.D.
Poliomyelitis	25	1.0 to 6.0	3.13±0.14	1.08
Ulcer	25	1.2 to 5.2	3.24±0.12	0.91
Coronary disease	25	1.2 to 6.2	3.77±0.13	0.99
Gall bladder disease	25	2.2 to 5.8	3.87±0.11	0.81

Table IV presents the differences of means of areola sizes after 24 hours' incubation. The mean values of the first two groups (poliomyelitis and ulcer) are again shown to be significantly smaller than those for the last two groups (coronary disease and gall bladder disease); this fact is indicated by the XPE values which are 3 or above in most instances.

TABLE IV

Differences of means and XPEs for intergroup comparisons of areola size, after 24 hours' incubation

	Difference and P.E.	XPE
Poliomyelitis/Ulcer	0.11±0.18	0.61
Poliomyelitis/Coronary	0.64±0.19	3.37
Poliomyelitis/Gall bladder	0.74±0.18	4.11
Ulcer/Coronary	0.53±0.18	2.94
Ulcer/Gall bladder	0.63±0.16	3.94
Coronary/Gall bladder	0.10±0.17	0.59

[8] The XPE is the difference between the means divided by the probable error of the difference. An XPE of 3 or more is considered to be statistically significant, for such a difference could only occur as a result of a sampling error in 4 cases out of 100. The larger the XPE, the smaller the possibility that such a difference could have arisen as a result of chance sampling. Thus, an XPE of 4 signifies that such a difference between the means could only occur as a result of chance sampling in 7 cases out of 1000 trials.

In a general way, the size of the areola at the 3½-hour and 24-hour incubation periods enables us to identify the culture as belonging either to the poliomyelitis-ulcer groups, or to the coronary-gall bladder disease groups. Since areola size seems only able to discriminate between two main pairs, further observational detail is required to differentiate more precisely the four original groups. It should be added at this point that especially characteristic of cultures from poliomyelitis susceptibles is the frequent presence at the edge of the explant of narrow areas of clumped dead cells and cellular debris. These areas we have called "collars." In Tables VA, VB, and VC further detailed observational (non-measurable) criteria concerning areola and cells are given.

This table of detailed observational criteria was based on the observations made on 328 cases representing the 4 disease groups. It was found, for example, that most of the poliomyelitis areolas were narrow while those of the gall bladder patients were wide. The ulcer and coronary cases usually fell in between these two extremes with the former group almost invariably possessing a slightly thinner areola than the latter. Similarly it was found that the cells from poliomyelitis patients were generally active while those from gall bladder patients were very quiet. Again, the peptic ulcer and coronary cases showed a less extreme picture.

The items in this table, then, represent the most frequently occurring or "typical" patterns. These criteria have been accepted as more or less standard for the four disease groups. When the table of criteria was finally established, each one of 290 cases was graded on a seven-point scale of agreement or lack of agreement with the standards. Thus, we desired to know how many of our poliomyelitis cultures, for example, exhibited all or only a few of the typical characteristics for that disease group. A rating of 7 (excellent) signified that the culture complied with the standard in all respects (8 or 9 items), the areola was within the proper size range, the cells displayed the prescribed amount and variety of activity, and all other characterizing points were fulfilled. A rating of 5 (good) was given to those cultures which complied satisfactorily with the standard in most respects (6 items), that is, still easily recognizable as matching its particular group pattern.

Cultures given a rating of 3 (fair) lacked many of the typical characteristics and were not easily recognizable as belonging to the proper pattern. Cultures classed 1 (unsatisfactory) were lacking in all but one or two items of the criteria for the specific disease group. The criteria used were 9 in number, 8 observational criteria and areola size.

In Table VII it is shown that 24 out of 84 poliomyelitis cultures possessed 8 or 9 criteria which have been judged typical for that disease group. Also, 34 out of 90 peptic ulcer cultures exhibited 8 or 9 criteria typical of that group. On the other hand, only 11 out of 47 coronary disease cultures and 10 out of 69 gall bladder disease cultures were rated 7 (excellent) on agreement between their observed criteria and those judged typical for these disease groups. Table VII also indicates that 69.0 per cent of the poliomyelitis cases conformed well (were rated 5 to 7) to the

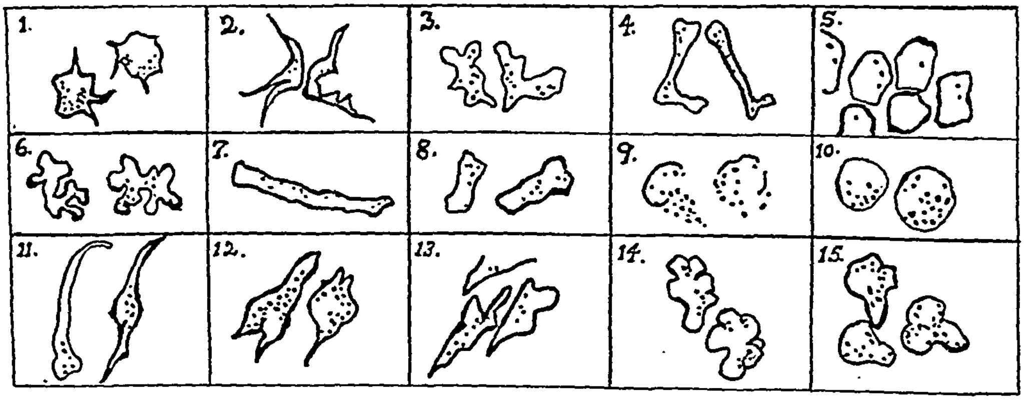

TABLE VA

Semi-diagramatic appearance of different cell types

1. Small, generally circular, with short, delicate spicules.
2. Irregularly branching form.
3. Leaf-like, rounded pseudopods.
4. Long, narrow, boot-like.
5. Square or polygonal; arranged like tiles.
6. Grotesque, "animal forms," angulated bodies, short, oval, and pointed pseudopodia.
7. Long, square-ended, rod-like forms.
8. Short, broad rods.
9. Ruptured polynuclears (ghosts).
10. Circular, inactive cells.
11. Long, club-shaped, or spindle-shaped cells.
12. Elliptical cells with one or two pointed pseudopods.
13. Medium-narrow, pointed cells, often lying in streaks.
14. Nobby, somewhat circular cells with small, blunt pseudopodia.
15. Pear-shaped cells with large, blunt, or broad-pointed pseudopodia.

TABLE VB

Preponderant cell forms

	At 3½ hours	At 24 hours
Poliomyelitis	1, 3, 10, 11, 15	2, 7, 8, 9, 11
Ulcer	1, 3, 6, 12, 15	3, occasionally 5, 6, 12, 13, 9, 10
Coronary	1, 3, occasionally 5, 4, 14	3, 4, occasionally 6, 9, 10
Gall bladder	1, 5, 8, occasionally 14, 15	same, with addition of 9

TABLE VC

Detailed observational criteria

	Areola thickness	Areola margin	Collars	Clumping	Cell concentration	Degree of motility	Pseudopodia	Cell shape
Poliomyelitis:	Narrow	Very irregular	Numerous	Rare	Light and scattered	Active	Short and bluntish	Rod shapes and linear forms
Ulcer:	Medium	Fairly regular	Rare	Rare	More dense	Less active	One or two pointed, medium long	Pear shaped pointed pseudopods
Coronary:	Slightly wide	Regular	None	Medium	Still more dense	Still less active	Many small nodular, or rounded	Ovoid rounded
Gall bladder:	Wide	Very regular	None	Almost constant	Very dense	Very quiet	Few—broad and normal	Rounded, flattish

TABLE VI

Rating scale in terms of typical criteria

A culture possessing	8 to 9 typical criteria	is rated 7	(excellent) on agreement
A culture possessing	7 typical criteria	is rated 6	(very good) on agreement
A culture possessing	6 typical criteria	is rated 5	(good) on agreement
A culture possessing	5 typical criteria	is rated 4	(fairly good) on agreement
A culture possessing	4 typical criteria	is rated 3	(fair) on agreement
A culture possessing	3 typical criteria	is rated 2	(poor) on agreement
A culture possessing	1 to 2 typical criteria	is rated 1	(unsatisfactory) on agreement

TABLE VII

Agreement between individual cultures and the standards for their respective groups for 290 cases
A 7-point scale is used in which a rating of 7 is excellent, 5 good, 3 fair, and 1 poor

	Poliomyelitis		Peptic ulcer		Coronary disease		Gall bladder disease	
culture rating	*number*	*per cent*	*number*	*per cent*	*number*	*per cent*	*number*	*per cent*
7	24	28.6	34	37.8	11	23.4	10	14.5
6	17	20.2	26	28.9	23	48.9	20	29.0
5	17	20.2	18	20.0	9	19.2	19	27.6
4	15	17.9	8	8.9	4	8.5	11	15.9
3	8	9.5	3	3.3	0	0.0	6	8.7
2	2	2.4	1	1.1	0	0.0	2	2.9
1	1	1.2	0	0.0	0	0.0	1	1.4
Total	84	100.0	90	100.0	47	100.0	69	100.0
5–7	53	69.0	78	86.7	43	91.5	49	71.1
1–4	26	31.0	12	13.3	4	8.5	20	28.9

standards for that disease group and were therefore judged to be typical poliomyelitis cultures. Similarly, 86.7 per cent of the peptic ulcer cases, 92.5 per cent of the coronary cases, and 71.1 per cent of the gall bladder cases were endowed with a sufficient number of criteria to be representative of their respective disease groups.

At this stage, it must be pointed out that if the poliomyelitis cultures, for example, were rated on the criteria for any of the other 3 disease groups, the agreement would be of a very low order. It is obvious, we believe, that the agreement ratings are for the most part mutually exclusive. That is, if a gall bladder culture is observed to possess a large percentage of the 9 criteria which we have judged to be typical for that disease group it is impossible for it to possess at the same time a large percentage of the criteria which are typical of any of the other 3 disease groups. There have been some instances in which gall bladder cultures, for example, possessed criteria which were more in agreement with those typical of the peptic ulcer or poliomyelitis groups and therefore could not be classified as typical gall bladder cultures. These overlaps, however, were found to be relatively rare. As a result of the above reasoning, we have found it possible to diagnose correctly slightly better than 70 per cent of specimens from unknown sources within the 4 groups.

DIFFERENTIAL CELL COUNTS

The approximate total number of cells per areola at 3½ hours of incubation was as follows: Poliomyelitis 6000 cells, peptic ulcer 17,000 cells,

PLATE I

FIG. 1. AREOLA ABOUT EXPLANTED FRAGMENT OF BUFFY COAT FROM BLOOD OF POLIOMYELITIS PATIENT, 3½ HOURS *in vitro*. × 25. Compare with Figures 2, 3, and 4.

FIG. 2. AREOLA ABOUT EXPLANTED FRAGMENT OF BUFFY COAT FROM BLOOD OF PEPTIC ULCER PATIENT, 3½HOURS *in vitro*. × 25.

FIG. 3. AREOLA ABOUT EXPLANTED FRAGMENT OF BUFFY COAT FROM BLOOD OF CORONARY PATIENT, 3½ HOURS *in vitro*. × 25.

FIG. 4. AREOLA ABOUT EXPLANTED FRAGMENT OF BUFFY COAT FROM BLOOD OF GALL BLADDER PATIENT, 3½ HOURS *in vitro*. × 25.

FIG. 5. CELLS IN CULTURE FROM BLOOD OF POLIOMYELITIS PATIENT, 24 HOURS *in vitro*. × 360. Note elongated cells.

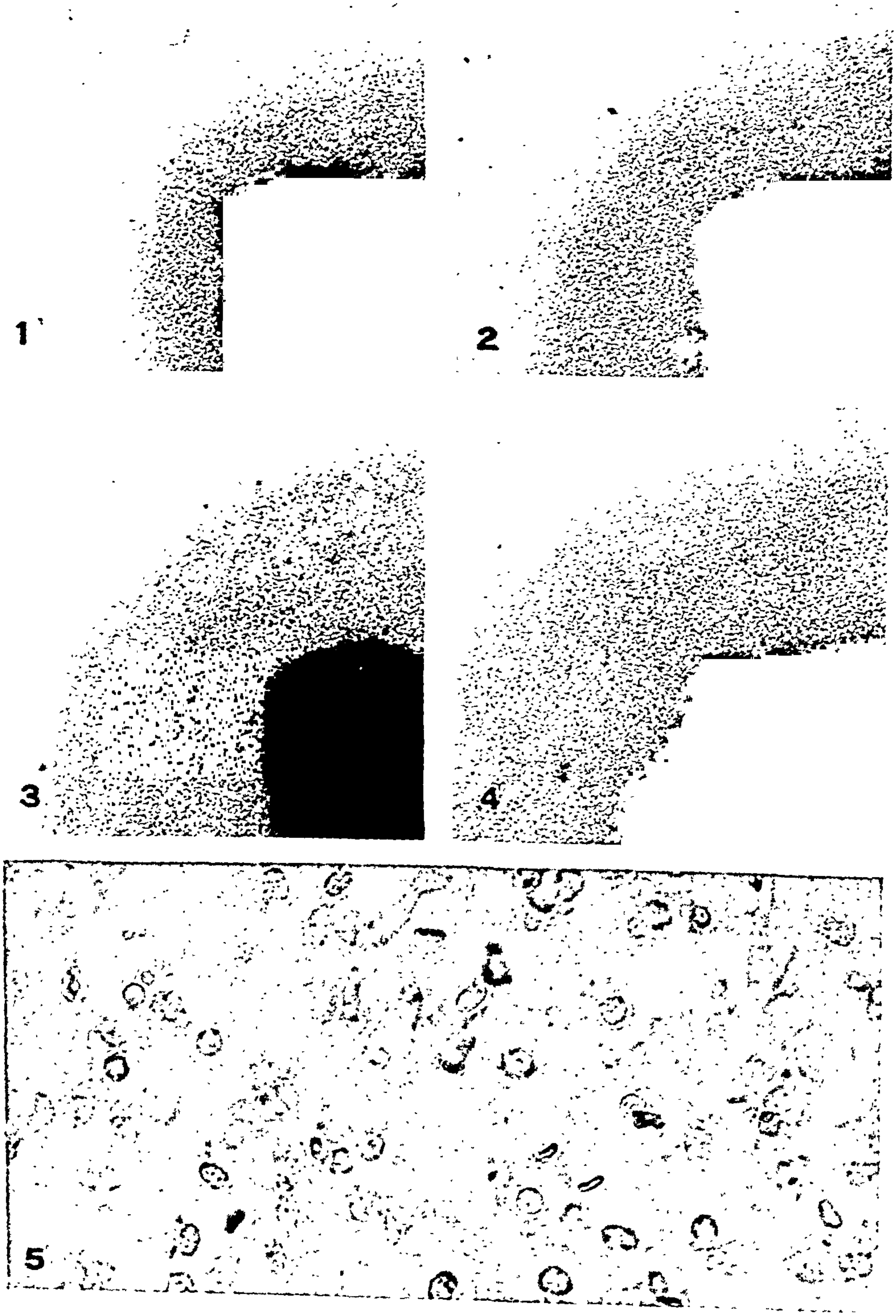

PLATE I

TABLE VIII

Differential counts of areolar cells in 4-hour cultures

	Poliomyelitis		Peptic ulcer		Coronary disease		Gall bladder disease	
	number	*per cent*	*number*	*per cent*	*number*	*per cent*	*number*	*per cent*
Neutrophils	3,300	55.0	9,740	57.3	16,550	59.1	21,760	64.0
Mononuclear cells	2,700	45.0	6,920	40.7	11,450	40.9	12,240	36.0
Eosinophils	0	0	190	1.1	0	0	0	0
Unidentified cells	0	0	150	0.9	0	0	0	0
Total	6,000	100.0	17,000	100.0	28,000	100.0	34,000	100.0

coronary disease 28,000 cells, gall bladder disease 34,000 cells. Cell counts at the 24-hour period still showed considerable variation among the disease groups. Approximations of total cellular content of the areolas at this stage were: poliomyelitis 53,000 cells, peptic ulcer 78,000 cells, coronary disease 91,000 cells, and gall bladder disease 121,000 cells.

It will be noticed that in the foregoing paragraphs attention has been directed only to the shapes and disposition of the leukocytes, without regard to their identity as neutrophils, monocytes, etc. By fixation and staining of whole cultures, interesting information may be brought to light concerning the proportional distribution of the hematological types.

Consequently, a set of 100 unselected cases, 25 patients from each of the 4 groups, has been drawn up in which differential counts of the hematological types of cells present in 4-hour and 24-hour cultures were made on specified sectors of the areolas, approximately 1000 cells per areola being counted.[4] At 24 hours it is impossible to distinguish between most monocytes and lympocytes since there are various changes in size and staining properties at this periol. The distinction on the basis of size is also founded on an arbitrary division. These two varieties have therefore been consolidated in the present report under the designation "mononuclear" cells. Most of the cells classed as unidentified are polynucleated forms, perhaps representing stages in cell multiplication. The differential counts are summarized in Tables VIII and IX.

It would appear that the differences in total and differential counts as shown in Tables VIII and IX may be definitely related to the different sizes and characters of the areolas as described above. It is also evident that the polynuclear neutrophils play the major part in these variations.

It is noteworthy that in the gall bladder cultures the absolute neutrophil count is 81,900 as against 12,150 for poliomyelitis; the respective mononuclear counts are 34,600 and 37,100. It is therefore obvious that, at least in the 24-hour culture, the difference in total count is altogether due to a difference in absolute neutrophil count.

DISCUSSION

In attempting to interpret the meaning of the foregoing observations, it should be pointed out that many of the distinguishing characteristics noted in living cultures may be attributed simply to the numbers and relative proportions of the various cell types. But, in addition, there are

[4] The number of cells was counted in each successive oil immersion field from the explant outward to the edge of the areola.

Total number of cells = 12.4 (3A + 7B + 11C + 15D + 19C etc.) in which A = sum of 1st 6 oil immersion fields
B = sum of 2nd 6 oil immersion fields
C = sum of 3rd 6 oil immersion fields
etc.

PLATE II

FIG. 6. CELLS IN CULTURE FROM BLOOD OF POLIOMYELITIS PATIENT. 24 HOURS *in vitro*. ×480. Compare with Figures 7, 8, and 9.

FIG. 7. CELLS IN CULTURE FROM BLOOD OF PEPTIC ULCER PATIENT, 24 HOURS *in vitro*. × 480.

FIG. 8. CELLS IN CULTURE FROM BLOOD OF CORONARY PATIENT, 24 HOURS *in vitro*. × 380.

FIG. 9. CELLS IN CULTURE FROM BLOOD OF GALL BLADDER PATIENT, 24 HOURS *in vitro*. × 480.

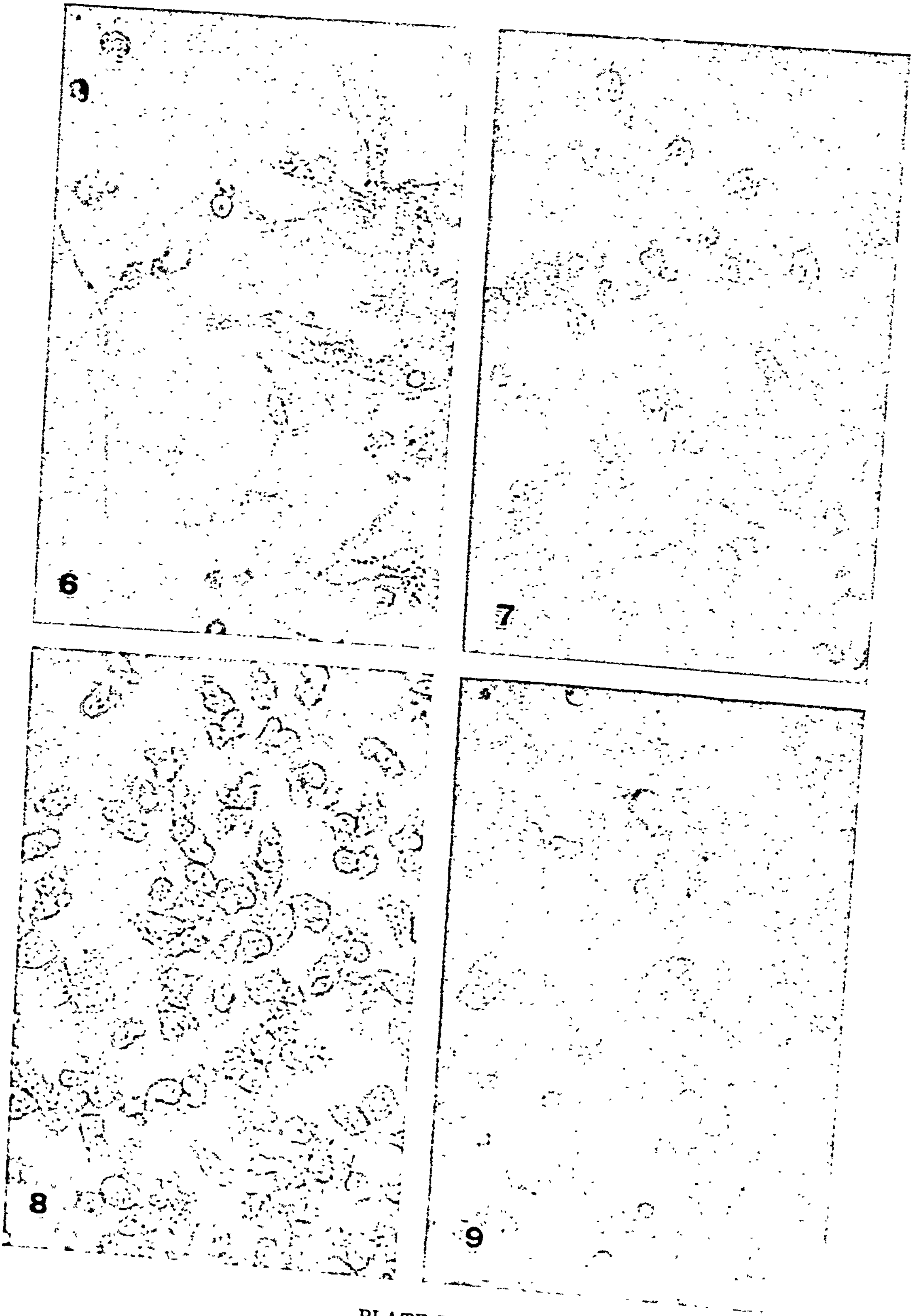

PLATE II

TABLE IX

Differential counts of areolar cells in 24-hour cultures

	Poliomyelitis		Peptic ulcer		Coronary disease		Gall bladder disease	
	number	*per cent*	*number*	*per cent*	*number*	*per cent*	*number*	*per cent*
Neutrophils	12,150	22.9	23,700	30.4	50,200	55.2	81,900	67.7
Mononuclear cells	37,100	70.0	50,300	64.5	35,400	38.9	34,600	28.6
Eosinophils	150	0.3	250	0.3	180	0.2	250	0.2
Unidentified cells	3,600	6.8	3,750	4.8	5,220	5.7	4,250	3.5
Total	53,000	100.0	78,000	100.0	91,000	100.0	121,000	100.0

other qualities which do not, to our knowledge, depend upon discernible hematological differences, but presumably upon some factor or factors which independently influence individual cell behavior. These qualities are expressed in the plastic aspect and modes of cellular locomotion. The obvious differences in general appearance between cultured cells from poliomyelitis and gall bladder patients, for example, or between those with ulcer and those with coronary disease are not solely quantitative. We are convinced that these living leukocytes assume in one case noticeably different plastic appearances and movements from those in another. This phenomenon may express certain qualities of constitutional physiology and may be analogous to the individual differences so easily seen in any whole living animal.

The immediate reason for decrease and disappearance of polymorphonuclear cells at an earlier hour in certain cultures than in others may, at this point, only be conjectured. A difference in fragility of the cells, reflected in their resistance to the mechanical forces involved in centrifugation and handling may be a factor. It is typical of the poliomyelitis cultures to find collars of disintegrated cells at the edge of the explant. But there is no doubt that variable energy in locomotion may be another. Differences in mononuclear cells in regard to aspect, mobility, and cell multiplication can undoubtedly be demonstrated. But it must not be overlooked that chemical and physical variation in the plasma may be partially or wholly responsible.

Much experimentation already reported in the literature has seemed to indicate that it is the medium in which the cells are maintained which primarily determines culture pattern (7, 11, 12). In the case of leukocytes cultivated in autogenous as well as homogenous plasma (plus 9-day chick extract), it is very likely that there are slight differences in chemical content of the plasma of the various individuals studied, which in some degree influence the behavior of the cells. In this connection, Baker reports that various proteolytic digestion products have different effects on the morphological appearance of the monocytes (13). However, the limited investigation of the media which we have made (namely crossing plasma and cells) points toward the conclusion that concerning *in vitro* behavior the plasma base does influence the pattern to some extent, but that this is a factor of less importance than normal variations among the cells themselves. It is impossible at this time to mark out clearly the interrelation of these two factors, but the question is of minor importance to the issue at hand. Since both plasma and cells in any of our preparations originate from the whole blood sample, the eventual manifestation of variation in terms of cell behavior may be regarded as a valid constitutional criterion regardless of the relative weight of factors determining that behavior. The most striking result of these studies is the vast difference displayed by comparing the patterns of buffy coat cultures from subjects of poliomyelitis and those of gall bladder disease. Less emphatic differential criteria are also discoverable in the samples from ulcer and coronary disease. But because these groups occupy intermediary positions the differences between the patterns of adjacent groups do not achieve such an extreme order of magnitude as in the first mentioned comparison.

The studies of gross constitution which have been based upon the observation and record of the four panels of personality (morphology, physiology, psychology, and immunity) have shown

that individual human types correlate with specific disease potentialities. From the study of the buffy coat cultures, similar correlations likewise appear. Thus, for example, in 217 of the 290 cases it has been possible to obtain evaluations for gross constitutional characteristics of the individual as well as culture ratings. These gross ratings are based on anthropomorphic, physiological, and psychological criteria recognized for subjects of each disease group. These standards have been described elsewhere (9, 10). A tabulation of these two sets of data follows (Table X).

Each of the two methods (gross or microscopic) shows an emphatic tendency to agree with the accepted standard for the method. The significance of the information derived from these two sources is not clear. But there is no question that the knowledge contributed by each points to the important conclusion that the personal identity of each individual is complete. It should be emphasized that by both methods some overlappings of types are found, but these are not sufficient to blur out the fundamentally basic type differences. In view of these observations, consequently, it would seem profitable to pursue further the cellular approach to constitutional research, and to include the cells of other body tissues in similar studies.

SUMMARY

1. Application of the technic of tissue culture to the problem of evaluating individual human constitution has demonstrated that each cell may display characters of aspect and behavior quite as specific as those which stamp the individual as a whole. Leukocytes from individuals of four disease groups—poliomyelitis, peptic ulcer, coronary disease, and gall bladder disease—were studied.

2. After 3½ hours, *in vitro* differences in areola size, form, and cellular content were demonstrated in the buffy coat cultures from poliomyelitis, peptic ulcer, coronary disease, and gall bladder disease.

3. Individual cultures were rated on a 7-point scale according to the degree to which they complied with standards for each of the four disease groups. Ratings of 5 (good) or above were assigned to 69.0 per cent of the poliomyelitis cases, to 86.7 per cent of the peptic ulcer cases, to 92.5 per cent of the coronary cases, and to 71.1 per cent of the gall bladder cases.

4. In 100 cases, differential counts were made on the areolar cells after 4 and 24 hours' incubation. Slight variation among the groups at 4 hours was exaggerated after 24 hours to display positive differences. In cultures from poliomyelitis patients, at one extreme, there was a preponderance of mononuclear cells and a scarcity of neutrophils, while in cultures from gall bladder patients, at the other extreme, were found a high neutrophil count and relatively few mononuclear cells. Cultures from the other two disease groups fell between these two extremes.

TABLE X

Agreement of culture ratings and gross constitutional ratings with standards for their respective groups for 217 cases
A 7-point scale is used in which a rating of 7 is excellent, 5 good, 3 fair, and 1 poor

Culture rating	Poliomyelitis				Peptic ulcer				Coronary disease				Gall bladder disease			
	Culture		Gross		Culture		Gross		Culture		Gross		Culture		Gross	
	number	*per cent*	*number*	*per cent*	*number*	*per cent*	*number*	*per cent*	*number*	*per cent*	*number*	*per cent*	*number*	*per cent*	*number*	*per cent*
7	19	29.2	10	15.4	23	37.7	18	29.5	11	27.5	5	12.5	8	16.7	11	22.9
6	13	20.0	19	29.2	19	31.1	24	39.3	18	45.0	13	32.5	14	29.2	7	14.6
5	12	18.5	30	46.2	12	19.7	12	19.7	8	20.0	16	40.0	12	25.0	8	16.7
4	13	20.0	5	7.7	5	8.3	4	6.6	3	7.5	2	5.0	7	14.6	12	25.0
3	7	10.8	0	0.0	1	1.6	1	1.6	0	0.0	4	10.0	5	10.4	6	12.5
2	1	1.5	0	0.0	1	1.6	2	3.3	0	0.0	0	0.0	1	2.1	2	4.2
1	0	0.0	1	1.5	0	0.0	0	0.0	0	0.0	0	0.0	1	2.1	2	4.2
Total	65	100.0	65	100.0	61	100.0	61	100.0	40	100.0	40	100.0	48	100.1	48	100.1
5 to 7	44	67.7	59	90.8	54	88.5	54	88.5	37	92.5	34	85.0	34	70.9	26	54.2
1 to 4	21	32.3	6	9.2	7	11.5	7	11.5	3	7.5	6	15.0	14	29.2	22	45.9

5. It is our opinion that the results warrant further study of the subject.

At this point, we take pleasure in extending our thanks and appreciation to Dr. A. P. Stout, Director of the Surgical Pathology Laboratory of the College of Physicians and Surgeons, and to his associate, Dr. M. R. Murray, for their cooperation in starting this research and working out adaptations of tissue culture technic for our special study. To Mrs. Irene Pogogeff who set up many of the early cultures, we are likewise indebted. It should be mentioned that the original grant of funds which started this work was given as a memorial to their daughter by Mr. and Mrs. E. H. Robins of Washington, D. C.

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SERUM ALBUMIN REGENERATION AS EFFECTED BY INTRAVENOUSLY AND ORALLY ADMINISTERED PROTEIN HYDROLYSATES [1]

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Observations on the ability of intravenously administered protein hydrolysates to regenerate plasma protein have been reported. One worker (1) employed an acid casein hydrolysate supplemented with tryptophane in acute hemorrhage in dogs and observed a very rapid, though small, increase in the plasma protein value. Later studies with pancreatic casein hydrolysate (2 to 4) indicated that the administration of adequate amounts by mouth or vein resulted in plasma protein regeneration. Using a papain casein digest, intravenously, in dogs made hypoproteinemic by plasmapheresis, others (5) have shown that plasma protein regeneration was effected.

Recently, there was observed (6) a small increase in the average plasma protein of 7 post-operative patients who were given an enzymic casein hydrolysate as the only source of nitrogen. This was coincident with a fall in the hematocrit value.

In the present study, we wished to determine, with as much exactitude as available methods permit, the comparative efficiency of different protein hydrolysates and their relative efficiency when given by mouth and by vein, in the regeneration of plasma albumin. Since wide differences have been shown (7) in the ability of different orally administered proteins to effect albumin regeneration, we felt it might be of interest to compare such proteins (as hydrolysates) given intravenously.

EXPERIMENTAL

Three enzymic protein hydrolysates were very kindly prepared and analyzed for us by Dr. K. S. Kemmerer of this laboratory. Casein, lactalbumin, and beef serum protein were used. The casein was acid-precipitated from skim milk.[2] Lactalbumin was a low ash, readily soluble, dried preparation.[3] Beef serum protein was prepared by dehydrating beef serum,[4] and, as supplied to us, contained about 15 per cent ash. To remove the major portion of salts, it was dissolved in water and precipitated by heat coagulation.

The proteins were enzymically digested in the usual way, using fresh pork pancreas as a source of enzyme. Digestion was complete in 7 to 10 days. The digest solution was heated, filtered, treated with a small amount of norite for decolorization, evaporated, and spray-dried to a fine powder. Table I gives comparative data on the hydrolysates.

To compare the nutritive value of the hydrolysate with the original protein, and thus to determine whether essential amino acids had been lost during the digestion and the technical processes thereafter, they were fed as the sole source of nitrogen in the basal diet described earlier for rats (8). The hydrolysates were fed on the basis of equivalence in nitrogen. Three levels of each equivalent to 2.4, 1.7, and 1.2 per cent nitrogen of the diet were fed. No less than 10 rats, approximately 50 grams in weight and 21 days old, equally divided as to sex, were placed on each diet. Growth was observed for 8 weeks.

For comparative measurement of serum protein regeneration, we used the technic developed by Weech and Goettsch (9). A mild degree of hypoproteinemia was produced by feeding their low protein diet to normal dogs for 3 weeks. During the subsequent week, the hydrolysate was incorporated in the diet to give a total intake of 0.34

TABLE I

Chemical characteristics of protein hydrolysates

	Casein	Lactalbumin	Serum protein
Total nitrogen, *per cent*	12.1	11.9	12.1
Amino nitrogen, *per cent*	8.2	8.7	9.0
Amino nitrogen after acid hydrolysis, *per cent*	10.2	11.3	10.6
Possible enzymic cleavage, *per cent*	80.0	77.0	85.0
Tryptophane, *grams per cent*	0.62	1.77	0.91
Ash, *per cent*	5.2	5.9	4.2
Moisture, *per cent*	4.9	2.7	2.2
Total N from pancreas, *per cent*	18.4	13.5	12.3
pH of 10 per cent solution	4.5	5.7	4.4

[1] Presented at the annual meeting of the American Society of Biological Chemists, Boston, March 31–April 4, 1942.

[2] Purchased from the Casein Corporation of America. Quality "H-I-P."

[3] Purchased from The Borden Company Research Division. Labco L A. 7-H-A.

[4] Purchased from Armour and Company.

grams nitrogen per kgm. body weight. The basal diet supplied 16 per cent of this total nitrogen. The diet supplied 80 calories per kgm. body weight. Plasma protein was determined by micro-Kjeldahl, and plasma albumin by precipitation from 22 per cent anhydrous sodium sulfate (10). The change in serum albumin during the week of supplementation was measured and was taken as the basis for comparison. For intravenous administration, the hydrolysate was given rapidly in 10 per cent solution sterilized by Seitz filtration. An average rate of 3 ml. per minute was tolerated but when rates in excess of this were given, vomiting frequently occurred.

RESULTS

The rat growth experiments are summarized in Table II. Complete comparative data are available only for the 1.7 and 1.2 per cent nitrogen intake levels since our supply of beef serum protein, and especially its hydrolysate, was limited. These lower intake levels are most useful, however, in estimating nutritive efficacy, since they correspond to approximately 14 per cent and 10 per cent of the hydrolysates, respectively. The growth on casein was obtained by averaging growth records for all samples of casein assayed. The data indicate that all 3 hydrolysates were very similar nutritively to the original proteins, with the possible exception of the casein hydrolysate. Thus, lactalbumin hydrolysate at the 1.2 per cent level was, if anything, superior to the original protein, and the gain in weight for serum protein and its hydrolysate was identical. For casein and casein hydrolysate, a 16 gram weight difference at the 1.2 per cent intake level is of questionable significance, since only one assay of the hydrolysate was made. However, it may reflect the relatively low tryptophane value of this particular lot. Since later observations on albumin regeneration in the dogs were not prejudicial to the sample, the question may be raised whether the requirements for growth are the same as for serum protein synthesis.

TABLE II

Average gain in weight of young rats when protein hydrolysates or the corresponding proteins were fed as the sole source of nitrogen for 8 weeks

Nitrogen source	Level of nitrogen intake		
	2.4 grams per cent	1.7 grams per cent	1.2 grams per cent
	grams	*grams*	*grams*
Casein	162.8[1]	59.2[1,2]	100.4[1]
Casein hydrolysate	130.2	58.0[2]	83.9
Lactalbumin	168.7	142.8	95.6
Lactalbumin hydrolysate	179.8	126.5	115.8
Beef serum protein		107.0	84.6
Beef serum protein hydrolysate		115.3	87.6

[1] Several separate lots of casein were fed to different groups of 10 rats each. The values are averages of 4 samples fed at the 2.4 per cent level, 5 fed at the 1.7 per cent, and 14 fed at 1.2 per cent level.
[2] Gain in weight for 4 weeks only.

TABLE III

Serum albumin regeneration effected by casein hydrolysate given intravenously and orally

Values expressed in grams per 100 ml. of plasma.

Dog No.	Total N intake grams per kgm.	Initial plasma albumin	Decline during depletion	Increase during supplementation	Assay value
		Intravenously			
9	0.345	2.55	−0.96	+0.58	+0.73
32	0.345	3.05	−0.94	+0.42	+0.57
21	0.345	3.12	−0.82	+0.07	+0.22
31	0.345	3.68	−1.20	+0.41	+0.56
37	0.345	3.20	−0.70	+0.04	+0.19
44	0.345	2.57	−0.54	+0.16	+0.31
		Average			+0.430±0.061
		Orally			
2	0.345	3.17	−1.18	+0.21	+0.36
43	0.345	3.20	−0.75	−0.06	+0.09
10 dogs *	0.324	(3.52)	−0.93	+0.23	+0.38
9 dogs *	0.370	(3.43)	−1.26	+0.19	+0.34
		Average			+0.348±0.022

* Taken from published data (8).

Tables III, IV, and V give the findings on serum protein regeneration for the 3 hydrolysates given orally and by vein. In these tables, we record the initial albumin value, its decline during 3-week depletion, its increase during regeneration, and the final assay value. This latter value is obtained by adding 0.15 gram per cent to the observed change in albumin (9), and takes account of the average decline that would have been observed during the fourth week if the supplement had not been given. The second column in Table III gives the total grams N intake per kgm. body weight. The small recorded differences in this figure are not significant. They were caused by

TABLE IV

Comparative serum albumin regeneration effected by lactalbumin hydrolysate given intravenously and orally (0.336 gram N per kgm.)

Values expressed in grams per 100 ml. of plasma.

Dog No.	Initial albumin	Decline during depletion	Increase during supplementation	Assay value
Intravenously				
9	2.65	−0.85	+0.46	+0.61
32	2.78	−0.44	+0.31	+0.46
37	3.44	−1.04	+0.06	+0.21
21	3.57	−0.73	+0.04	+0.19
44	3.58	−0.56	+0.08	+0.07
	Average			+0.308±0.067
Orally				
2	2.92	−0.62	+0.27	+0.42
35	3.25	−0.97	+0.27	+0.42
60	3.07	−0.40	+0.17	+0.32
43	3.27	−0.65	−0.02	+0.13
	Average			+0.322±0.046

assumptions as to the protein equivalence of the nitrogen in the hydrolysate.

For casein hydrolysate given orally, an assay value of 0.348 was obtained. This is an average of 21 hypoproteinemic dogs, 19 of which have been previously reported (8). One investigator (11) reported 0.425 as the assay value for unhydrolyzed casein and this value does not differ significantly from our value for the hydrolysate given by mouth. When intravenously given, the average assay value for 6 dogs was 0.430, which again does not differ significantly from either casein or casein hydrolysate fed by mouth.

Five dogs were used for the intravenous injection of lactalbumin hydrolysate and 4 served as the oral controls. The average assay value, orally, was 0.322 and, intravenously, 0.308. These values must be regarded as identical, and within the range of values observed for casein hydrolysate. Six of the 9 dogs had been used for the previous studies with casein hydrolysate, and a comparison of individual performance on different supplements showed closely similar performance on both.

Serum protein hydrolysate intravenously gave an assay value of 0.287 (Table V), but orally, an assay value of 0.660. The intravenous value is not significantly different from 0.308 value for lactalbumin hydrolysate. But an assay value of 0.660 for the protein hydrolysate given by mouth is difficult to interpret. It is unfortunate that our supply of this material was exhausted and that further data could not be obtained. Aside from the fact that there are only 4 observations on oral feedings, it should be noted that the first 2 dogs were penned together and got into a fight on the day before the depletion value was recorded. The dog that showed the greatest assay value was badly beaten in the fight. This may or may not be a pertinent factor. In spite of this, a value of 0.660 is in agreement with that reported (11) for dried beef serum (assay value 0.739) but the limited number of observations impels acceptance of the value as provisional.

DISCUSSION

As a result of the work of others (7, 11 to 16), it is recognized that ingested proteins differ widely in their ability to regenerate plasma albumin. Our work has been entirely with protein hydroly-

TABLE V

Serum albumin regeneration effected by beef serum protein hydrolysate given intravenously and orally (0.334 gram N per kgm.)

Values expressed in grams per 100 ml. of plasma.

Dog No.	Initial plasma albumin	Decline during depletion	Increase during supplementation	Assay value
Intravenously				
9	2.91	−0.93	+0.53	+0.68
31	2.92	−0.96	+0.09	+0.24
32	4.05	−1.51	+0.19	+0.34
2	3.32	−0.48	−0.32	−0.17
35	3.05	−0.86	+0.19	+0.34
44	3.71	−1.00	+0.14	+0.29
	Average			+0.287±0.075
Orally				
2	3.04	−0.66	+0.55	+0.70
35	2.71	−1.25	+0.79	+0.94
9	3.38	−0.72	+0.43	+0.58
31	2.97	−0.59	+0.27	+0.42
	Average			+0.660±0.074

sates rather than with whole protein. We have determined the effectiveness of hydrolysates of different proteins and the efficiency of the same hydrolysate given by vein and by mouth in albumin regeneration. All the hydrolysates given by vein resulted in plasma albumin synthesis. In 6 dogs, casein hydrolysate gave an assay value of 0.43 gram per cent; lactalbumin hydrolysate, in 5 dogs, gave 0.308 gram per cent; and serum protein hydrolysate in 6 dogs gave 0.287 gram per cent. Statistically, there is no difference between the high and low values and we must regard the 3 hydrolysates as equivalent in their ability to regenerate albumin.

When the assay values for the same hydrolysate given by mouth and by vein are compared, we can again elicit no statistically significant difference. The greatest difference was recorded for serum protein hydrolysate given by these two routes. The P value for the difference is 0.053, which strongly suggests a significant difference. However, the limited number of dogs fed by mouth, the fact that the animals were penned together, and the finding that neither lactalbumin hydrolysate nor casein hydrolysate showed any difference in regeneration, whether given by mouth or by vein, makes us feel that the same is likewise true for the serum protein hydrolysate.

The finding that intravenously administered hydrolysates of good proteins are equally effective in serum albumin regeneration is clearly different from the conclusions of others that intact proteins fed by mouth are quite widely different in effectiveness. Our hydrolysates were the equivalent of the intact protein as measured by the growth of experimental animals (Table II). In explanation of this difference, we suggest the following hypothesis.

It has been shown that for the establishment of nitrogen balance, all essential amino acids must be present in the circulation at the same time (17). If this is true, it is not unlikely that differences in the rate of protein hydrolysis and in the rate of absorption of the amino acids from the gastrointestinal tract may greatly influence the effectiveness of a particular protein in serum albumin regeneration. That proteins are hydrolyzed at different rates and to different extents, and that amino acids are readily freed from some protein linkages but that some form resistant groupings, are well-recognized facts (18, 19). Further, it was shown (20) that there are differences in the rate of absorption of the different amino acids.

It follows that the composition of the amino acid mixture which may eventually appear in the blood may differ widely from the actual composition of the ingested protein. Therefore, in measuring the effect of an ingested protein by means of plasma albumin regeneration, one may be measuring not an intrinsic difference in proteins based on ultimate composition but the net resultant of varying rates of enzymic hydrolysis and absorption from the intestine.

When a hydrolysate instead of intact protein is given orally, one eliminates the necessity for intestinal hydrolysis and all the amino acids are immediately available for absorption. By intravenous administration, the further factor of differing rates of absorption is eliminated. Since we observed that plasma albumin is regenerated to an equal extent whether the protein hydrolysates were given by mouth or by vein, it would seem that the rate of intestinal hydrolysis of proteins is the factor which has determined recorded differences in the ability of intact protein of good nutritive quality to regenerate plasma albumin.

CONCLUSIONS

Enzymic hydrolysates of casein, lactalbumin, and beef serum protein, which are nutritively equivalent to the original proteins, are equally effective in the regeneration of plasma albumin in hypoproteinemic dogs, whether given orally or intravenously. The significance of these findings is discussed.

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THE EFFECT OF TESTOSTERONE COMPOUNDS UPON THE NITROGEN BALANCE AND CREATINE EXCRETION IN PATIENTS WITH THYROTOXICOSIS [1, 2]

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The tendency to increased urinary nitrogen excretion in thyrotoxicosis has been well demonstrated (1 to 5). The presence of hypercreatinuria in thyrotoxicosis has recently been reviewed and confirmed (6, 7).

In view of the constancy of these two findings, and their importance in the pathologic physiology of Graves' disease, it seemed reasonable to postulate that an agent which would tend to reverse or inhibit both of these processes, might have some beneficial effect upon the disease. Testosterone propionate has been shown to exert a protein-anabolic and a creatine-retaining effect in animals (8) and in individuals with other clinical syndromes (9 to 12). It has also been shown to reduce the creatinuria of experimentally induced hyperthyroidism of monkeys (13).

Methyl testosterone, like testosterone propionate, causes protein anabolism but, unlike testosterone propionate, causes extreme creatinuria (14). Furthermore, methyl testosterone has been reported to exert a significant calorigenic effect, in contrast to testosterone propionate, which has little or no action of this kind (8, 9, 15, 16).

We were interested, therefore, in determining the effects of these two compounds upon the nitrogen and the creatine excretion, and upon the clinical status of patients with thyrotoxicosis.

MATERIALS AND METHODS

Studies on 3 thyrotoxic patients are reported in this paper. All of the patients were stabilized before the metabolic studies were begun upon a diet that was higher in protein, calories, and vitamins than the usual requirements of normal individuals. This diet was maintained throughout the period of observation. Activity was kept at an approximately constant level throughout the experimental period.

Urinary nitrogen was determined by the micro-Kjeldahl method on aliquots of 3-day pools. The nitrogen content of the stool was considered to be one-tenth of the intake. This assumption was checked in one patient during the period of greatest nitrogen retention, and found to be essentially correct. Urinary creatine and creatinine were determined every third day, on an aliquot of that day's specimen (17). Excretion of 17-ketosteroids was determined on aliquots of 3-day urine pools by a modification (18) of the original method. The basal metabolic rate was determined at frequent intervals, using the Dubois standard. Body weight was recorded at least once during each 3-day period. Serum constituents were determined by standard laboratory procedures.

After a suitable number of control periods, the patients were given courses of varying amounts of testosterone propionate intramuscularly or methyl testosterone, orally. The former was employed in a concentration of 25 mgm. per ml. of sesame oil; the latter in tablets, each containing 10 mgm. of the steroid.

With the exception of patient P. C. (Case 3), none of the patients reported in this series had received iodine at any time prior to the period of metabolic study.

RESULTS

Case 1, M. M., U#362811 (Lab. #23209), an unmarried female, aged 28, gave a 2-months history of increasing weight loss in spite of a goodly food intake, sweating, palpitation, and nervousness. Her menstrual history was normal. Physical findings included a moderately prominent, diffusely enlarged thyroid gland, with bilateral thrill and bruit. There was little or no exophthalmos, and no other eye signs. Her skin was moist and flushed; she had a definite tremor, and was generally hyperreactive. The mean of two basal metabolic rates, done before the institution of therapy, was +35 per cent. The metabolic and other data obtained on this patient are shown in Figure 1.

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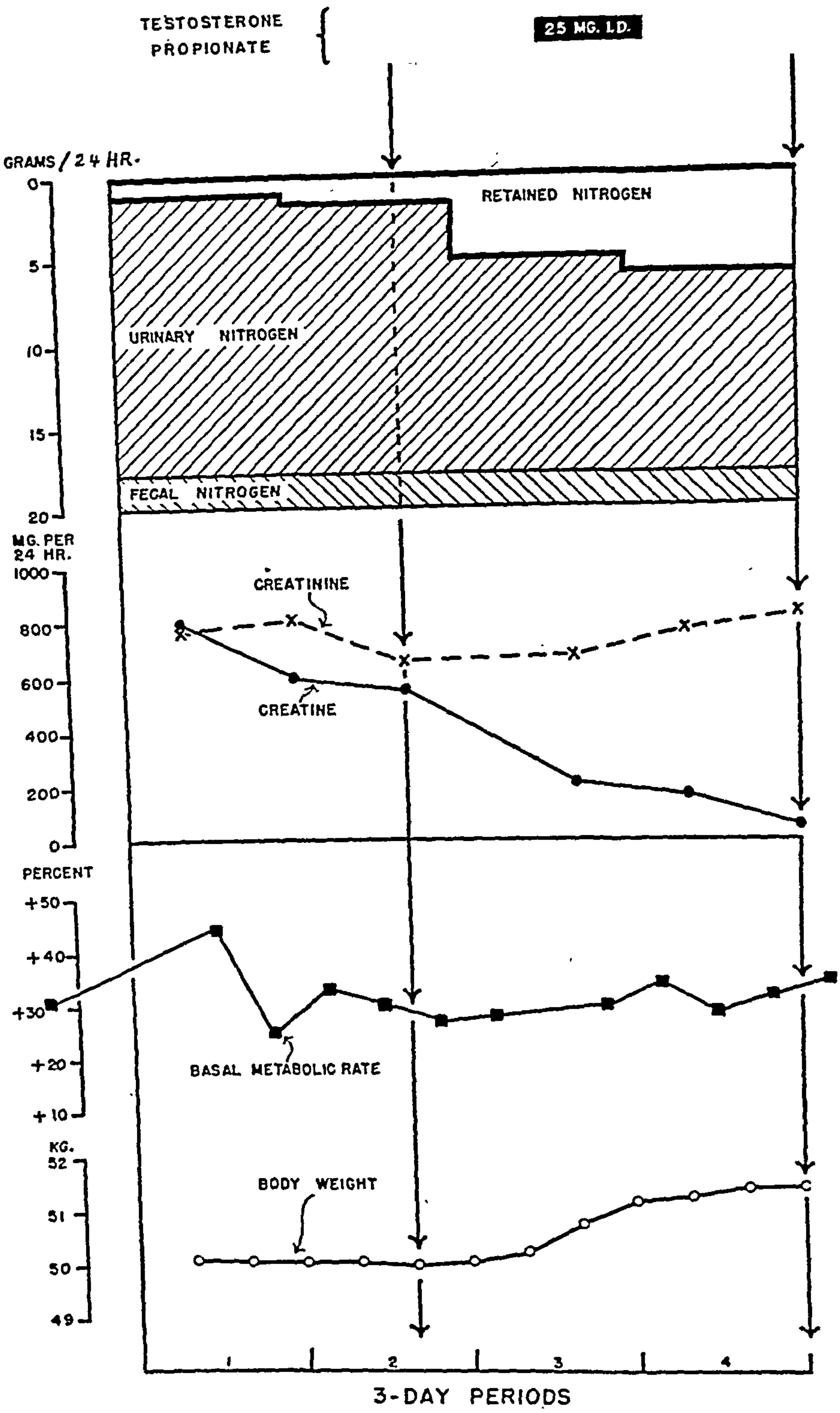

FIG. 1. EFFECT OF TESTOSTERONE PROPIONATE ON PATIENT M. M. (CASE 1)

In this and subsequent charts, nitrogen intake (protein ÷6.25) is represented by the interval between the "O" line and the bottom line (in this case the "20" line). The fecal and urinary nitrogens are so labelled. If the line representing the summation of urinary and fecal nitrogen lies below the "O" line, the individual is in positive balance. Correspondingly, if the output line rises above the "O" intake level, the individual is in negative nitrogen balance. All metabolic data are reduced to values per 24 hours rather than per period.

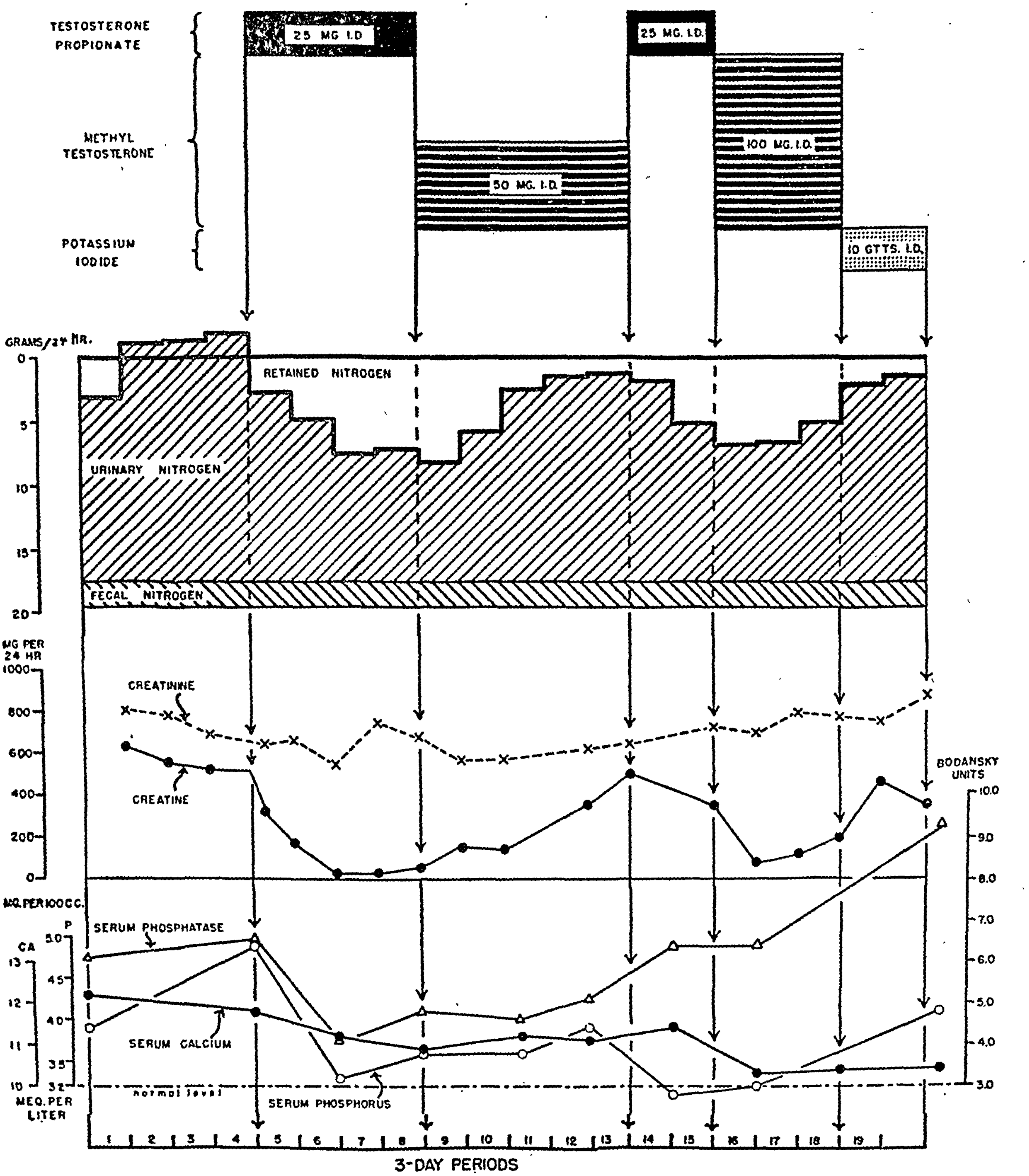

FIG. 2A. EFFECT OF TESTOSTERONE PROPIONATE AND METHYL TESTOSTERONE ON PATIENT H. L. (CASE 2)

In spite of the brief control period in this patient, the increase in nitrogen retention, the gain in weight, and the decrease in creatinuria, induced by testosterone propionate therapy, are quite apparent. Her basal metabolic rate did not change significantly.

Case 2, H. L., U※369099 (Lab. ※23307), a schoolgirl, aged 14, gave a story of weight loss in spite of a large food intake, rapid growth, nervousness, tremor, sweating, and quite marked enlargement of the neck of 1 year's duration. At the time of the metabolic study, the menses had not appeared, although pubic hair and breast development were present. One sister had been operated upon for hyperthyroidism at the age of 16. Physical findings included a prominent, diffusely enlarged thyroid gland,

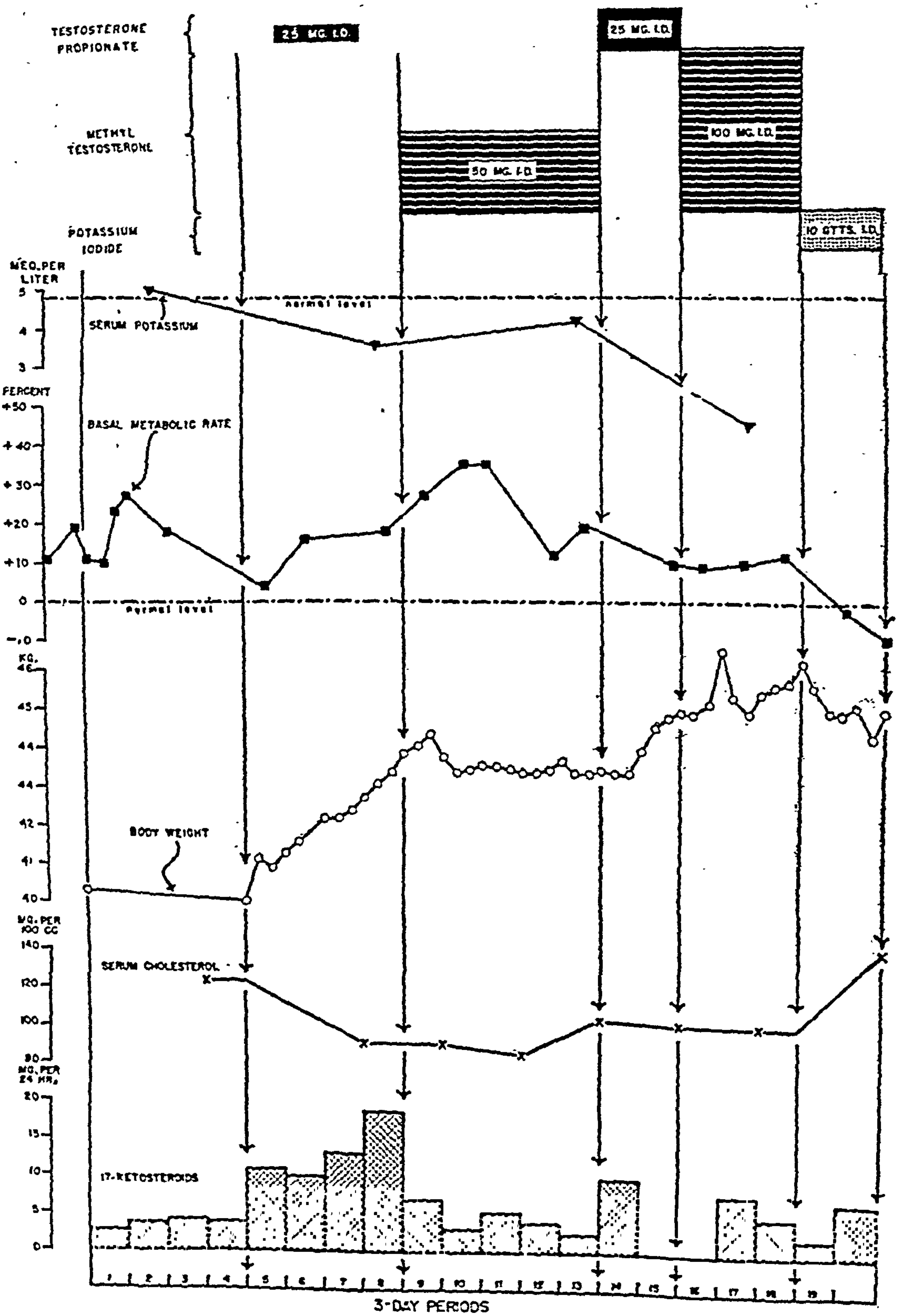

Fig. 2b. Case 2 continued

with bilateral thrill and bruit; slight exophthalmos without lid lag or disturbance in convergence; pulse rate of approximately 100 per minute. Fine tremors of tongue and hands and hyperkinesis in general were readily observable. Her palms were warm and moist. The mean of 7 pre-treatment basal metabolic rates was + 17 per cent.

After stabilization upon the diet, her nitrogen and creatine excretions were determined for 4 periods, during which time she had extreme creatinuria, lost a little weight, but was approximately in nitrogen balance (Figures 2A and B). On testosterone propionate (25 mgm. daily), she retained progressively more nitrogen, gained weight, and put out decreasing quantities of creatine. With methyl testosterone (50 mgm. daily), her nitrogen balance became progressively less positive, she failed to gain weight, and excreted increasing amounts of creatine in the urine. There was a suggestion of an increase in the basal metabolic rate and clinically she became irritable and uncooperative. Testosterone propionate therapy was then resumed, with results almost identical with those obtained during the initial administration: marked nitrogen retention, rapid gain in weight, and decreased creatinuria. Following this, she received methyl testosterone, 100 mgm. daily, for 3 periods. The data indicate that this dosage of methyl testosterone had a less sustained effect upon the nitrogen retention than the 25 mgm. dosage of testosterone propionate. Furthermore, the larger dosage of methyl testosterone induced only a moderate gain in weight and increased the excretion of creatine. Clinically, she seemed much more toxic, but this finding was not reflected in the basal metabolic rate.

Steroid therapy was then stopped, and she was placed on potassium iodide. Her basal rate dropped immediately to minus levels, but in spite of this she retained progressively less nitrogen. (This "rebound" on the basis of our experience with other patients is probably much less than would have occurred had she received no potassium iodide.)

The serum calcium and phosphorus values, which had been somewhat elevated prior to therapy, tended to become normal during the administration of testosterone compounds. The serum phosphatase, which was moderately elevated prior to therapy, fell to normal levels during the initial period of testosterone propionate administration, but tended to rise during further treatment. The one value obtained during potassium iodide medication was elevated well above the pre-treatment level. The serum potassium dropped to subnormal levels during treatment.

The 17-ketosteroid excretion, which was relatively low prior to treatment for a girl of this age, increased during testosterone propionate administration (18), but dropped to pre-treatment levels with methyl testosterone. It is interesting that her last value while on potassium iodide was more than double her initial pre-treatment level.

Case 3, P. C., U#369235 (Lab. #23326), a 45-year-old male, gave a 9-months history of extreme weakness, nervousness, and weight loss despite a goodly food intake, and an almost lifelong history of emotional instability, tremor, and profuse perspiration. His thyroid gland was moderately enlarged. A bruit could be discerned on the right side. Exophthalmos was questionable; there was no lid lag, and but slight disturbance in convergence. A tremor of the hands and feet could be readily demonstrated. His skin was quite moist and warm. Before admission to the metabolic ward, he had received potassium iodide for 2 months, and had had a single oral administration of radioactive iodine two and one-half months previously. His basal metabolic rate on admission was + 42 per cent. Since he was fully iodinized but definitely thyrotoxic, we continued him on potassium iodide throughout his metabolic studies.

We desired in this patient to determine the effects of different dosages of testosterone propionate, and of methyl testosterone, and particularly to discover the relative dosages of each required to produce a similar degree of protein anabolism. Also we wished to see whether the adverse clinical effects of methyl testosterone, previously noted on patient H. L. (Case 2), would be manifest in this patient.

He was placed, accordingly, on the following program: (*1*) 4 periods of no treatment (except potassium iodide); (*2*) 4 periods of 12.5 mgm. of testosterone propionate daily; (*3*) 4 periods of 100 mgm. of methyl testosterone daily; (*4*) 4 periods of 25 mgm. of testosterone propionate daily; (*5*) 4 periods of 50 mgm. of testosterone propionate daily; (*6*) 6 periods of 100 mgm. of testosterone propionate daily; and (*7*) 4 periods of no treatment (except potassium iodide).

The findings during these various regimes are shown in Figures 3A and B.

The findings on the previous patients were confirmed. Specifically, a very positive nitrogen balance was produced with testosterone propionate in all dosages used, and the degree of nitrogen retention was either increased or maintained at a constant level throughout the period of administration of the compound; methyl testosterone, on the other hand, while unquestionably causing nitrogen retention, failed to exert the sustained effect characteristic of testosterone propionate. Thus, the nitrogen balance became progressively less positive throughout the period of administration. In this patient, testosterone propionate completely stopped the excretion of creatine in the urine, while methyl testosterone caused a marked creatinuria. During the time that the patient received methyl testosterone, he became so toxic and unmanageable that we were tempted to discontinue the medication before the 4 periods were completed. Conversely, we felt that he received definite clinical benefit from the testosterone propionate administration. It should also be noted that he had been impotent for many months. Even with the very large dosage of androgens employed, no objective or subjective effects upon the genitalia were induced.

With the diet employed, he lost weight rapidly prior to testosterone propionate administration, but was

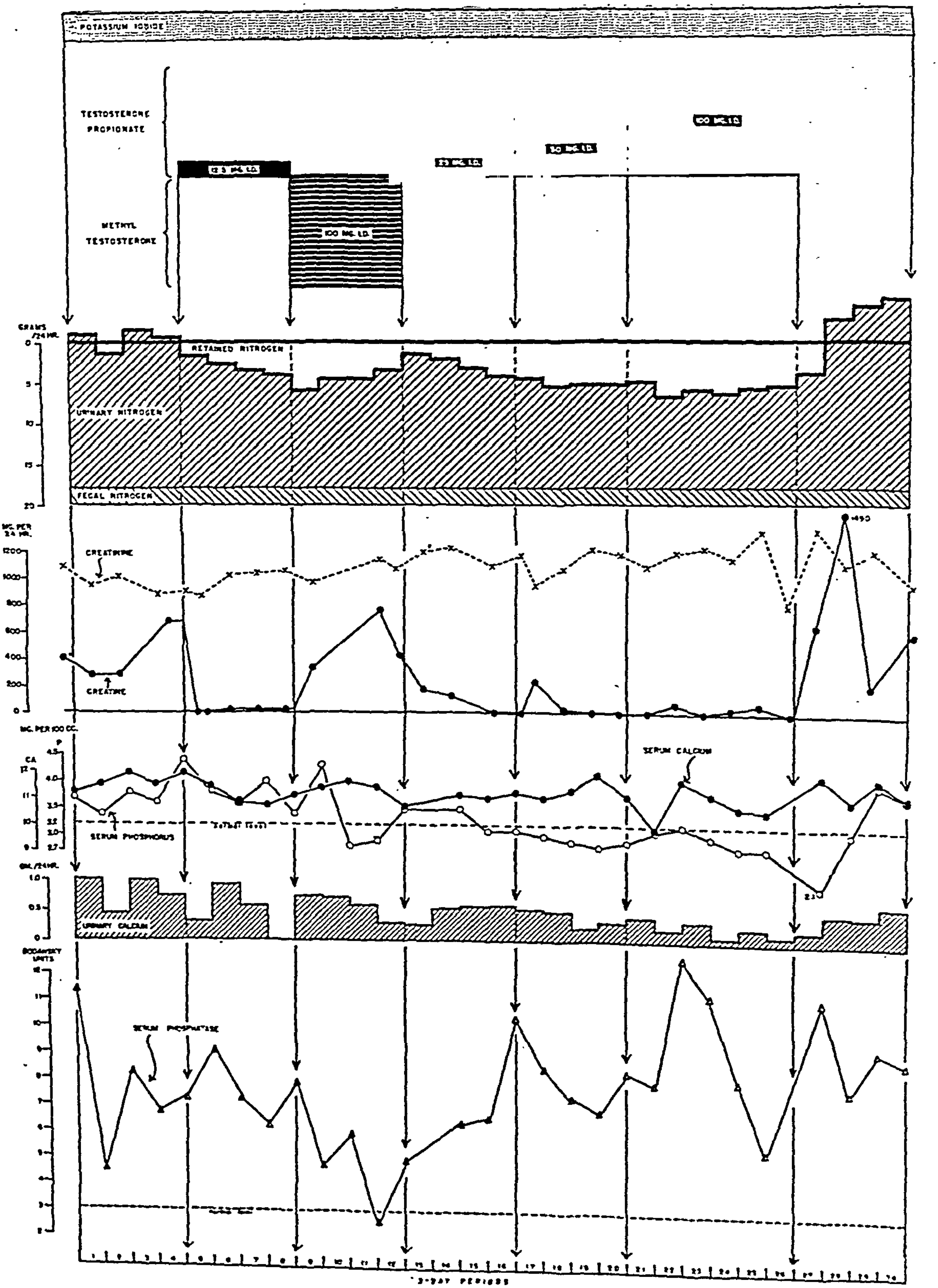

FIG. 3A. COMPARATIVE EFFECTS OF TESTOSTERONE PROPIONATE AND METHYL TESTOSTERONE ON THYROTOXIC PATIENT P. C. (CASE 3)

Potassium iodide, gtts. 15, given daily throughout metabolic study.

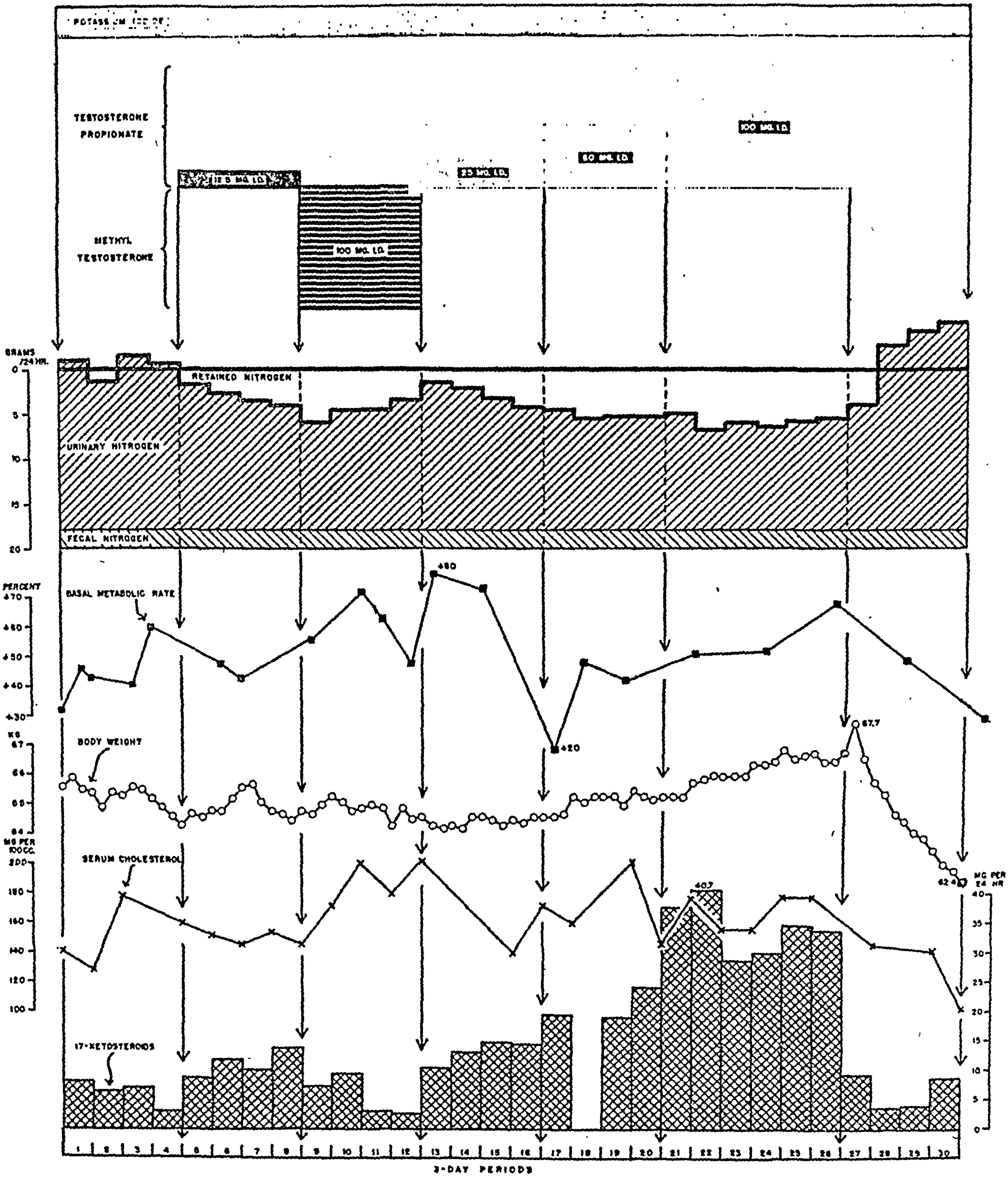

FIG. 3B. CASE 3 CONTINUED

approximately in nitrogen balance. During the time that he received testosterone propionate and methyl testosterone, his weight remained fairly constant. Following cessation of testosterone-compound therapy, he went into a very negative nitrogen balance and showed a rapid weight loss and an intense creatinuria.

As in patient H. L. (Case 2), there was a tendency to depression of the serum calcium and inorganic phosphorus levels during the period of steroid administration. In the case of the latter element, the effect seemed to be proportional to the dosage employed. The serum phosphatase showed extreme degrees of fluctuation, the interpretation of which is not apparent. It fell to a normal value during methyl testosterone administration, but was more

or less elevated at all other times. The serum cholesterol level showed no significant change.

The alterations observed in the 17-ketosteroid excretion were comparable to those obtained in patient H. L. (Case 2.) The pre-treatment level was very definitely subnormal.

DISCUSSION

Effect of thyroid hormone on nitrogen metabolism

The inter-relationships existent or thought to be existent between the thyroid hormone and nitrogen metabolism are very complex, and will be enumerated with little or no discussion. In the first place, it was thought (4) that the level of thyroid hormone in the body controlled the amount of "deposit protein". This contention and the validity of the concept of "deposit protein" itself will not be considered further here. Secondly, the thyroid hormone is intimately connected with growth and hence nitrogen retention. Finally, as regards the data here presented, the most pertinent fact is that most patients with thyrotoxicosis exhibit a loss of body protein. It is almost certain that this loss is connected with the increased energy output. That there is not a specific effect of the thyroid hormone on protein catabolism was well demonstrated by investigators (19) who showed that hyperthyroid patients on high calorie, high carbohydrate, high fat, low protein diets had normal "minimal nitrogen excretions". Presumably, the nitrogen depletion in such cases has resulted from a catabolism of protoplasm at some time during the illness in order to meet the increased energy requirements.

Effect of testosterone compounds on nitrogen metabolism

Regardless of the cause of this depletion, it is clear from the data in this study that therapy with testosterone propionate overcomes this depletion. It was to be expected that testosterone therapy would cause a positive nitrogen balance in hyperthyroid patients, since it is clearly established that testosterone compounds cause marked nitrogen retention in patients with many other disorders (8 to 12). It also seems quite definite that such nitrogen retention indicates the synthesis of protoplasm, inasmuch as phosphorus, potassium, and sulfur are simultaneously retained in approximately the ratios in which these substances are associated with nitrogen in protoplasm (20, 21).

These anabolic effects of testosterone compounds have been established on patients receiving an adequate caloric intake. However, in the present study, the intake of patient P. C. (Case 3) proved to be: (*a*) adequate in nitrogen, since the patient remained in nitrogen equilibrium during the control periods, but (*b*) inadequate in calories, since the patient lost weight rapidly during the control periods in spite of the greater-than-normal caloric content of the diet. The results in this case indicate that testosterone therapy is able to induce anabolism of protoplasm in a patient on an adequate-nitrogen, inadequate-calorie intake. Furthermore, it has been demonstrated that testosterone propionate therapy can reestablish nitrogen equilibrium in an obese individual on an intake inadequate in both nitrogen and calories (21). These findings suggest that testosterone compounds can stimulate anabolism of protoplasm even in the presence of a demand for gluconeogenesis for energy purposes.

Qualitative differences between methyl testosterone and testosterone propionate

The apparently qualitative differences between the effects of testosterone propionate and methyl testosterone on anabolism of protoplasm in thyrotoxicosis are of interest, especially since we have failed to note marked differences of this sort when the two compounds have been used alternately in patients with other clinical syndromes (20, 21). The calorigenic properties of methyl testosterone (8, 9, 15, 16) or its effect upon creatine excretion (9, 10, 14) suggest themselves as factors that may account for these differences.

However, the calorigenic property can probably be dismissed. Certainly in patient H. L. (Case 2), the objective and subjective clinical manifestations could not be accounted for on the basis of change in basal metabolic rate. The same may probably be said for patient P. C. (Case 3) although his basal metabolic rate was quite high during and immediately following methyl testosterone administration.

The creatinuria-inducing property of methyl testosterone requires more serious consideration,

for the amount of nitrogen lost from the body as creatine during the administration of this compound proves to be far too small to account quantitatively for the decrease in protein anabolism that occurred at the same time.

Evidence has been produced (22) which suggests that the creatinuria induced by methyl testosterone probably results from an increased formation of creatine with resultant increased excretion, rather than from an increased excretion of pre-formed creatine. According to one author (23), creatine is formed in the body by the methylation of guanidoacetic acid (glycocyamine), and in this process the methyl groups are supplied by choline and methionine and the guanidoacetic acid from a reaction between glycine and arginine. The formation of creatine at any moment, therefore, depends on the amounts available of choline, and 3 amino acids (glycine, arginine, and methionine); the quantities of these substances, in turn, are governed by two factors: (*1*) the amounts ingested, and (*2*) the amounts derived from metabolism of protein, either through increased catabolism or decreased anabolism. Creatine, once it is formed, does not appear to enter into any further chemical reaction involving transfer of nitrogen; it is simply stored in muscle or eliminated in the urine. Any factor which increases creatine in the urine, therefore, tends to deplete the body stores of these 4 substances.

Two of these substances, methionine and arginine, are essential amino acids. It has been shown (24) that a positive nitrogen balance cannot be maintained in the absence of methionine. These findings have subsequently been confirmed (25). Similar experiments (26) have shown that arginine, while not having a pronounced effect on the nitrogen balance, is necessary for maximum growth and normal reproduction. Hence, adequate amounts of methionine and arginine are needed for both protein anabolism and creatine formation; these processes compete, in a sense, for these two amino acids. Creatine, itself not an indispensable food constituent, thus contains an organic chemical grouping indispensable to the body; and loss of creatine through the urine represents loss of material needed for protein anabolism. In hyperthyroidism, the increased energy metabolism and the creatinuria would tend to deplete the stores of methionine and arginine.

In the patients with hyperthyroidism under discussion, methyl testosterone induced sustained creatinuria but only transient protein anabolism. A plausible explanation for the action of methyl testosterone in these patients was advanced recently by Dr. Ephraim Shorr (27). It was suggested that methyl testosterone altered the competition for methionine and arginine between protein anabolism and creatine formation in favor of the latter process, so that as the supply of these amino acids began to run low, creatine formation was maintained at the expense of decreased protein anabolism. Testosterone propionate, on the other hand, by reducing creatine formation, tended to conserve these amino acids for use in protein anabolism.

Calcium metabolism

We have only fragmentary data on the effect of testosterone propionate on the disordered calcium metabolism characteristic of hyperthyroidism (28). In patient P. C. (Case 3), it should be noted that the high calcium excretion in the urine was reduced under therapy with testosterone propionate. This change will not be discussed until further data have been collected.

Serum potassium

The serum potassium values in patient H. L. (Case 2) are of interest. It will be observed that the value dropped to subnormal levels during testosterone administration (see Figure 2B). This is in accord with recent findings (29). These investigators noted with testosterone therapy a lowering of the serum potassium level without an increase in the urinary potassium excretion and without any clinical manifestations of potassium deficiency; this was in contrast to the hypokalemia and hyperkaluria resulting from desoxycorticosterone acetate administration. Presumably, the testosterone-induced hypokalemia is merely a reflection of the migration of large amounts of potassium into the newly formed protein tissue.

The possible clinical usefulness of testosterone propionate in the management of thyrotoxicosis

Extreme weight loss occasionally occurs in thyrotoxic patients in whom the usual compensa-

tory appetite increase fails to take place. Such patients probably have depletion of body protein. The finding of liver damage in fatal cases of thyrotoxicosis (30 to 32) is in accord with the concept that there is a causal relationship between diminished hepatic stores and liver damage (33, 34). A therapeutic agent capable of causing a rapid replacement of such losses should prove of considerable value in the preoperative management of patients with marked wasting. Since testosterone propionate produces no significant depression of the basal metabolic rate, the simultaneous administration of iodine or thiouracil might induce a more favorable response than either agent alone. Patient P. C. (Case 3) does not represent the optimal in such a combination, inasmuch as he had already passed his period of maximal iodine response when testosterone therapy was begun.

Analysis of the nitrogen retention in relation to dosage in patient P. C. (Case 3) (Figure 3A) suggests that 12.5 mgm. of testosterone propionate daily produced as great an effect as 100 mgm. daily. However, this may not be true, inasmuch as the larger dosages of testosterone propionate were not used until he had already retained a large amount of protein. Had the 100 mgm. dosage been used initially, the immediate effect might have been greater. In any event, the definite nitrogen retention induced by 12.5 mgm. daily in this very toxic patient suggests that this dosage is adequate.

SUMMARY AND CONCLUSIONS

1. Since thyrotoxicosis is characterized by an increase in the urinary excretion of nitrogen and creatine, and by a decrease in body weight, and since testosterone propionate has the opposite effect on these 3 variables, studies have been carried out on the metabolic effect of testosterone propionate and methyl testosterone on 3 patients with thyrotoxicosis.

2. In these patients, testosterone propionate induced a markedly positive nitrogen balance and caused a weight gain; these effects were obtained in patients whose diet was constant, and even in individuals whose caloric intake was less than their caloric expenditure.

3. Methyl testosterone had a similar initial effect on the nitrogen balance, but its effect was not sustained; the difference between methyl testosterone and testosterone propionate may possibly be attributed to the calorigenic effect of the former, or more probably to its different effect on creatine metabolism (*vide infra*).

4. Testosterone propionate decreased the hypercreatinuria which characterizes thyrotoxicosis; methyl testosterone increased it. It is suggested that methyl testosterone may increase creatine formation at the expense of protein anabolism.

5. In the one patient with thyrotoxicosis in whom calcium studies were carried out, there was, with testosterone propionate therapy, a striking reduction in the hypercalciuria characteristic of thyrotoxicosis.

6. The effect of testosterone propionate in reducing the serum potassium level was confirmed in one patient.

7. Testosterone propionate improved the clinical status of the thyrotoxic patients; methyl testosterone, *on the contrary, aggravated their toxicity*. The latter drug is probably contraindicated in this disease.

8. Testosterone propionate may prove to be a useful therapeutic adjunct in preparing for operation those thyrotoxic patients who have sustained marked weight loss, with emaciation and muscle wasting. A daily dosage of 12.5 mgm. is probably adequate. This should be given, of course, in addition to, not in place of, whatever drug is used to reduce the metabolic rate,—iodine or thiouracil.

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AUTOHEMAGGLUTININS[1]—"COLD AGGLUTININS"

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Until cold agglutinins were reported in the 1943 serum of primary interstitial pneumonia patients (1), the phenomenon was considered rare. These findings were promptly confirmed by investigators who also observed low titers for cold agglutinins in respiratory infections without pneumonia (2 to 4). Cold agglutination was first accurately described in 1903 (5, 6) and was first found in association with bronchopneumonia in 1918 (7). 1910 Except for trypanosomiasis (8), primary interstitial pneumonia is the only reported condition commonly associated with cold agglutinins. Single or small groups of cases of a variety of other diseases have been found to have cold agglutinins. Among these diseases are Laennec's 1931 (9) and syphilitic (10, 11) 1936-37 cirrhosis of the liver, 1938-41 hemolytic anemia (12, 13), paroxysmal hemo- 1935 globinuria (14, 15), peripheral vascular disease 1937 (16), benzene poisoning (17), pneumonia of unusual type (18), infectious mononucleosis (13), 1943 pernicious anemia, hyperproteinemia, and severe pneumonias (11). Of practical as well as etiological interest is the patient with cold agglutinins (19) who developed symmetrical gangrene of the fingers and toes following exposure to cold. Cold agglutinins have also been found in cats (20) and have been produced experimentally in rabbits by blood letting (21 to 24). The agglutinin is a globulin (7) which moves with the gamma groups on electrophoretic analysis (25).

The investigation of an institutional outbreak of atypical pneumonia and epidemically related respiratory infections was in progress (26) when the presence of cold agglutinins in the sera of atypical pneumonia patients was reported (1). Because the cold agglutinin test offered a laboratory means of extending our observations on the relationship between atypical pneumonias and common respiratory infections, the present study was undertaken. Patients with a number of other illnesses, acute and chronic, and a group of normal subjects were also studied for control purposes. A report on the occurrence of cold agglutinins in normal subjects, in persons with respiratory infections, and in a number of general hospital patients follows.

METHODS

Collection of blood. The stability of cold agglutinins is influenced by the method of collection and storage. We found that uniform results were best obtained when blood was drawn under sterile conditions, allowed to stand at room temperature until the clot retracted (4 to 24 hours) in order not to adsorb agglutinins on the patients' cells, and the separated serum stored aseptically at 4° C. in a tightly stoppered tube. The highest titers were obtained when the blood was tested on the day collected. The titer fell gradually over the first 3 weeks and more rapidly when the serum was repeatedly warmed for sampling. Serum stored at —70° C. in sealed glass ampoules maintained its titer for 6 months.

The agglutination test. Serial dilutions (1:5, 1:10, 1:20, etc.) of serum in 0.2 ml. of saline were mixed with 0.2 ml. of an 0.5 per cent suspension of freshly drawn, 3 times washed, normal group O human red cells (same donor throughout) and stored for 18 hours at 5° C. Shorter periods of chilling and higher temperatures did not give as uniform results. Readings were made by shaking the tube 3 times, firmly enough to make a silk-like suspension in negative tests. Agglutination was observed with the unaided eye before a 40-watt light bulb. The degree of agglutination was recorded as 1 to 5, 1 representing just visible agglutination and 5, a solid clump of cells. All tests were warmed, and the reversibility of agglutination confirmed. The results were recorded as serum dilutions. The titers reported are 1+ end-points. Since the titers regularly show an average fall of 1+ per tube as the end-point is reached and since prozones were not observed, results were uniform. Where serial tests were done on individual patients every 2 or 3 days through the course of an infection, the end-points rose and fell in smooth curves. Tests were done in groups of 20 to 70 in order properly to relate known positives and negatives to the results of the previous test on the same patient.

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RESULTS

Cold agglutinins in normal subjects. In March, April, June, and July of 1943, groups of 25 student nurses were found to have no cold agglutinins.

In September of 1943 (Figure 1), serum for cold agglutinins was drawn from 27 student nurses, 2 weeks after they first entered training. This was done at the time of their admission physical examination and x-ray of the chest. No one of these had had atypical pneumonia. One nurse gave a history of a "cold," 2 weeks before. One nurse had a chronic sore throat. Each of these had a cold agglutinin titer of 1:40. Five nurses had fresh "colds" or "coughs"; none of these had cold agglutinins. Twenty nurses were well; 5 had cold agglutinin titers, 4, 1:10, and one, 1:40. During September, October, and November, the 20 well nurses developed 17 respiratory infections, and the nurse with the sore throat also had a cold. Four of the 5 nurses who had cold agglutinins without a respiratory infection when first tested subsequently developed coughs or colds. The fifth remained well, maintaining a cold agglutinin titer of 1:40 throughout the fall, later returning to negative. Of the group with fresh respiratory infections and of those who subsequently had respiratory infections, 4 out of 5 developed a cold agglutinin titer of 1:5 or more (Table I). In November, 10 of the 27 had or were recovering from a cold or cough. Six of the 10 had cold agglutinin titers; 3 were 1:5, 1 was 1:10, and 2 were 1:40. Individuals with cold agglutinins during an infection have since gradually returned toward negative. On the other hand, the nurse with the chronic low-grade sore throat

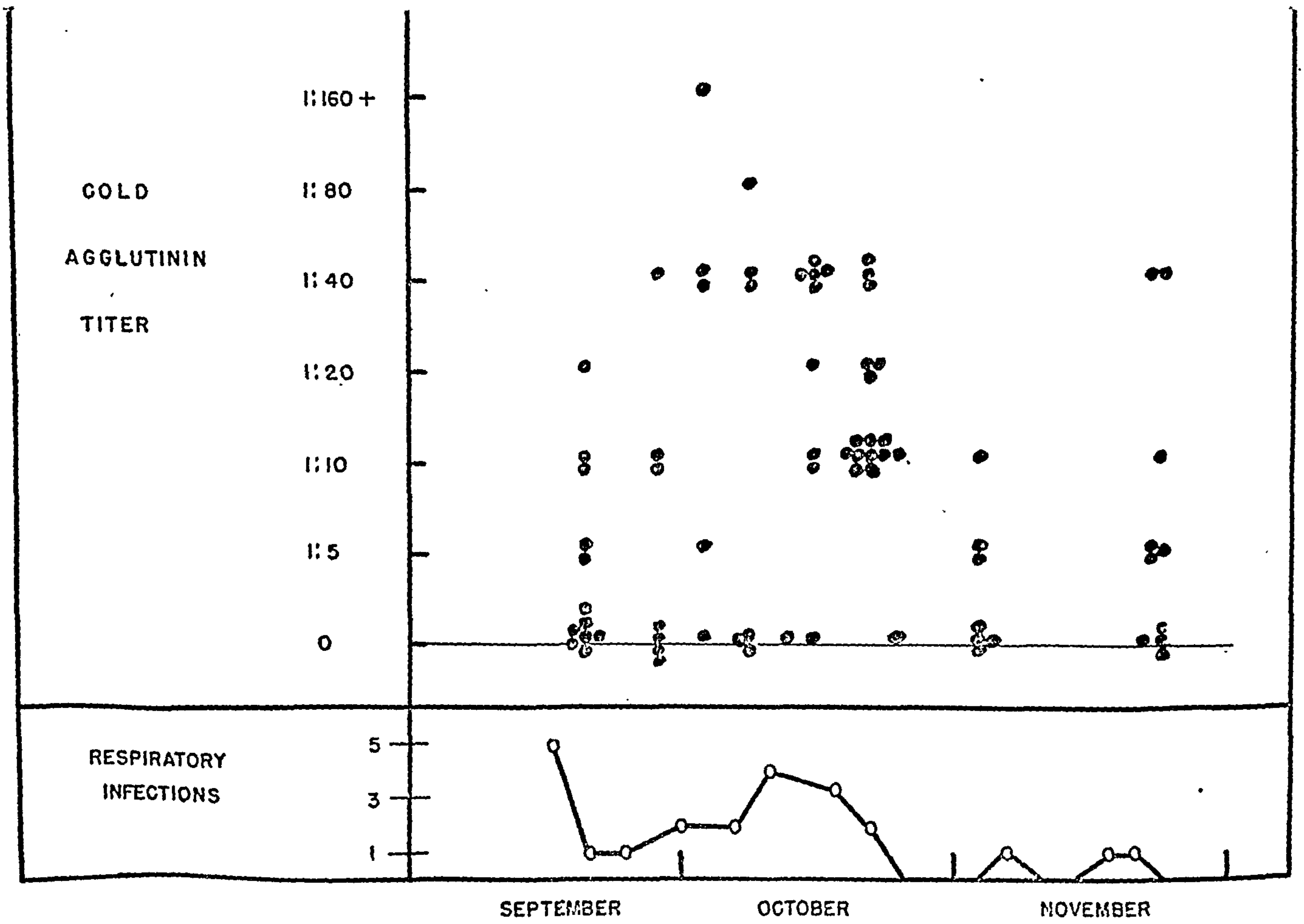

Fig. 1. A Record of 73 Cold Agglutinin Tests on 27 Nurses Collected from the Student Nurse Groups Discussed in the Text

Ten had colds, 13 tracheobronchitis, and 1 cold agglutinins without symptoms. The number of respiratory infections indicates those whose onset was within the preceding 5 days. The increased number of infections correlates roughly with the elevated cold agglutinin titers.

TABLE I

Maximum cold agglutination titers *

Disease	0	1:5	1:10	1:20	1:40	1:80	1:160+	Total no. of patients
Atypical pneumonia	4	1	3	6	6	8	18	46
Tracheobronchitis	7	0	6	2	4	7	1	27
"Colds"	3	2	4	0	3	1	1	14
Influenza (complement fixation test positive)†	3	0	1	2	2	1	0	9

Summary: Atypical pneumonia—32 out of 46 patients had cold agglutinin titers of 1:40 or more (69 per cent); 18 of 46 patients had titers of 1:160 or more (39 per cent).

Tracheobronchitis—12 out of 27 patients had cold agglutinin titers of 1:40 or more (45 per cent).

"Colds"—5 out of 15 patients had cold agglutinin titers of 1:40 or more.

Influenza—9 patients with clinical influenza occurring during a recognized epidemic had influenza A complement fixing antibodies rising during the course to 1:64 or higher.

Two patients who had cold agglutinin titers during influenza had had a previous attack of tracheobronchitis also accompanied by similar cold agglutinin titers. Neither of the 2 had cold agglutinin titers during the interval before developing influenza.

* Each patient had 2 or more sera tested, one of them 14 days or later after the acute onset of his disease.

† The author wishes to acknowledge the kind assistance of Dr. John F. Enders of the Harvard Medical School in whose laboratory the complement fixation tests were performed.

has maintained a titer of 1:40 during the winter (4 months).

In October of 1943, 22 second and third-year student nurses, also reporting for their physical check-ups and chest x-rays, were studied in the same fashion as the above group. Six of these had cold agglutinin titers of 1:20 or higher (included in Table I). A careful history showed that all but one of these with cold agglutinins had had a "cold" or tracheobronchitis within the previous 2 weeks. The one exception was admitted to the hospital 2 days later with a classical tuberculous pleurisy with effusion. This student nurse has since been followed and found to have a titer rising to 1:40 during the height of symptoms and a return to normal in convalescence. In the others with respiratory infections, the titers have steadily returned toward negative.

Hereditary cold agglutinins have been postulated (7). In Table II are records on 3 patients who have maintained high cold agglutinin titers for long periods of time despite minimal stimuli. If one of these patients had been tested for cold agglutinins between infections, he might well have been considered an example of hereditary cold agglutinemia.

TABLE II

Cases of recurring cold agglutinins

	Date	Titer	Day of disease
Miss M. E.			
Atypical pneumonia	4/20/43		
German measles	5/15/43		
	5/25/43	1:40	10th
	6/24/43	1:40	40th
	7/20/43	1:40	66th
Begin daily exposure, atypical pneumonia	8/25/43		
Tracheobronchitis, mild	9/3/43		
	9/17/43	1:160	14th
	9/21/43	1:80	18th
	9/28/43	1:80	25th
Very slight cough	10/6/43		
Malaise, temperature 101.4° 1 day	10/7/43	1:320	2nd
Slight cough	10/10/43	1:40	5th
Well	10/28/43	1:40	23rd
Slight cough	11/3/43	1:80	29th
	11/15/43	1:20	41st
Gastro-intestinal upset, temperature 100.4° 1 day	11/18/43		
	11/22/43	1:80	48th
Well	12/6/43	1:80	61st
Chronic cough 1 month			
Heavy exposure to atypical pneumonia December to present	2/16/44	1:160	30th
Dr. R. B.			
Atypical pneumonia, mild	6/23/43	1:20	7th
	6/26/43	1:40	10th
Well	7/1/43	1:40	15th
	7/7/43	1:40	21st
	7/10/43	1:20	24th
Tracheobronchitis, mild	9/20/43		
	10/4/43	1:80	14th
Still slight cough	10/15/43	1:20	25th
Very slight cough	11/10/43	1:5	51st
Very slight cough	12/31/43	1:10	102nd
Miss J. C.			
Onset, atypical pneumonia	5/2/43		
	5/4/43		2nd
	5/6/43	1:10	4th
	5/9/43	1:40	7th
Afebrile	5/12/43	1:40	10th
	5/15/43	1:40	13th
	5/18/43	1:80	16th
Cough gone, patient well	6/7/43	1:40	36th
	7/1/43	1:40	60th
	7/26/43	1:20	85th
Begin heavy exposure, atypical pneumonia	8/14/43		
	9/22/43	1:40	39th
	9/29/43	1:160	46th
	11/3/43	1:40	81st
	11/15/43	1:80	93rd
Slight "flu"; ambulatory	12/1/43		
	12/3/43	1:80	111th
	12/14/43	1:80	122nd
	1/12/44	1:20	151st
Roommate "cold" 4 days before	2/16/44	1:160	183rd

TABLE III

Cold agglutinins in other diseases

Disease	Comment	Number of patients	Maximum titer
	Virus diseases		
Rubella	1 month after atypical pneumonia	1	1:80
	1 month after scarlet fever	1	1:40
	Mild	1	1:20
	Mild	1	1:10
		1	1:5
		2	
		7	
Measles	With bronchopneumonia	1	1:40
	Severe	1	1:40
	Mild	2	
		4	
Chicken pox	Mild	1	1:40
	Mild	1	1:5
		2	
Mumps		1	
	With meningitis	1	1:20
	With meningitis	1	1:5
	Moderately severe	1	1:40
		4	
Ornithosis		1	1:80
		2	
		3	
Trachoma		1	1:80
Trachoma		1	1:40
		2	
Lymphopathia venereum		1	1:10
Lymphopathia venereum		1	
		2	
Meningo-encephalitis		2	
	Bacterial diseases		
Beta hemolytic streptococcus	Pharyngitis	1	1:80
	Pharyngitis	2	1:5
	Pharyngitis	3	
	Otitis media chronic	1	1:80
	Severe surgical scarlet fever	1	
	Severe scarlet fever	1	1:80
	Severe scarlet fever	1	1:20
		1	1:20
	Moderate scarlet fever	1	1:10
	Moderate scarlet fever	3	1:5
		7	
		22	

TABLE III—(*Continued*)

Disease	Comment	Number of patients	Maximum titer
	Bacterial diseases—(Continued)		
Pneumonia	Bronchopneumonia	1	1:5
	Bronchopneumonia	5	
	Severe type II; empyema; serum treatment	1	1:10
	Pneumonia with empyema	1	1:40
	Pneumonia with lung abscess	1	1:160
	Pneumonia with lung abscess and empyema	1	1:40
	Type IV and ? atypical pneumonia	1	1:10
	Classical lobar	1	1:20
	Classical lobar	6	
		18	
Tuberculosis	Childhood type simulating atypical pneumonia	1	1:5
	Epituberculosis	1	
	Pleurisy with effusion	1	1:40
	Pleurisy with effusion	1	1:20
		1	1:10
		1	
	Pulmonary with superimposed bronchopneumonia	1	
	Minimal apical	1	1:5
	Minimal apical	1	
	Miliary	1	
	Widespread pulmonary	1	1:10
	Widespread pulmonary	1	1:10
		1	1:5
		1	
		14	
Endocarditis	Subacute bacterial endocarditis, para-influenza	1	1:40
	Subacute bacterial endocarditis, streptococcus viridans	2	
	Acute staphylococcus aureus	1	
		4	
	Blood diseases		
Acute lymphatic leukemia		1	1:40
Acute lymphatic leukemia		1	
Chronic hemolytic anemia with jaundice		1	1:160
Chronic myeloid leukemia		3	
Terminal myeloid leukemia		1	1:280,000
Polycythemia-leukemia		1	
Untreated severe pernicious anemia		1	

TABLE III—(*Continued*)

Disease	Comment	Number of patients	Maximum titer
Blood diseases—(Continued)			
Infectious mononucleosis		2	1:80
		1	1:20
With jaundice		1	1:40
With glands and sore throat		2	1:20
With glands and sore throat		4	
		10	
Miscellaneous diseases			
Asthmatic bronchitis		1	1:20
		1	1:10
		2	1:5
		4	
Acute rheumatic fever			
Rheumatic fever with chorea		1	
Exacerbation, chronic		1	
Acute rheumatic fever		5	
		7	
Pulmonary edema		1	1:5
Pulmonary edema		1	
		2	
Chronic bronchiectasis and pulmonary fibrosis		1	
Extensive metastasis to lung		2	
Carcinoma bronchus, advanced		2	
Acute hepatitis		1	1:40
Acute hepatitis		1	1:80
		2	
Mild hepatitis with jaundice		1	1:20
Mild hepatitis with jaundice		1	1:10
		1	1:5
		1	
		4	
Catarrhal jaundice		1	1:5
Catarrhal jaundice		2	
		3	
Terminal cirrhosis		1	
Hepatitis without jaundice		1	
Ulcerative colitis		1	
Syphilis		1	1:5
Syphilis		20	
		21	

TABLE III—(*Continued*)

Disease	Comment	Number of patients	Maximum titer
Miscellaneous diseases—(Continued)			
Chancroid		1	1:10
Disseminated lupus erythematosus		2	1:80
		2	
		4	
Sarcoid		1	
Scleroderma		1	1:5
With esophageal stricture		1	1:20
		2	
Acute focal myocarditis		1	1:10
Mikulicz syndrome		1	
Multiple myeloma		1	
Bronchial asthma		1	1:10
Bronchial asthma		2	
		3	
Erythema nodosum		1	1:20
Erythema nodosum		4	
		5	
Insulin allergy		1	1:40
Following massive intravenous typhoid for fever therapy		1	
Severe ivy poisoning		1	
Allergic rash		1	

DISCUSSION

Why cold agglutinins should be frequent among patients in a general hospital, as is indicated in the tables, is not clear. A sensitive method and the listing of only the maximum titer chosen from multiple tests on each subject are contributing reasons. Higher readings also may have resulted from careful collection of serum without chilling blood below room temperature before storing serum (3). Multiple tests on a single person have also demonstrated the persistence of cold agglutinins for months following illness, seasonal changes of titer with or without accompanying common respiratory infections, and rises in titer with different infections. Whether these ex-

amples represent an endemic disease due to a specific agent, an institutional infection, or a chronic individual infection is not known. That low titers occur with minor respiratory infections, however, is known (2). Although cold agglutinins have not been found with lobar pneumonia and febrile conditions treated with sulfonamides (1) nor in normal subjects (3), controls taken from the variety of patients here presented have not been reported.

In the present patients, serum cold agglutination titers up to 1:40 were found in association with a number of acute infectious diseases due to viruses and bacteria, as well as associated with many miscellaneous conditions, including hepatitis, leukemia, and idiopathic hemolytic anemia. Similarly, a great many normal subjects were found to have cold agglutinin titers within the same range. These normals, however, usually had a history of or showed signs of an infection antedating the presence of cold agglutinins in their sera. Furthermore, these normals usually lost their cold agglutinins with the passage of time or later developed an infection in turn followed by a further serum cold agglutinin response.

In the present study, serum cold agglutinin titers of 1:160 or more were uncommon. Atypical pneumonia, the common cold, severe lobar pneumonia with empyema, chronic idiopathic hemolytic anemia, and myeloid leukemia were the only conditions in which so high a titer was present. Eighteen out of 46 atypical pneumonia patients had a serum cold agglutinin titer of 1:160 or higher.

In our group, a cold agglutinin titer of 1:40 or more was present in many conditions. Thirty-two out of 46 atypical pneumonia patients, 12 out of 27 tracheobronchitis patients, 5 out of 14 patients with "colds", and scattered patients with rubella, measles, chicken pox, mumps, ornithosis, trachoma, streptococcal infections, pneumonia, tuberculosis, endocarditis, hepatitis, allergy, leukemia, and infectious mononucleosis, had cold agglutinin titers of 1:40 to 1:80. From these findings, it would seem that the significance of serum cold agglutinins depends not only on the clinical findings in the patient but also upon the amount of agglutination. Although cold agglutinins were present in atypical pneumonia serum more often than in the other diseases studied in this report, only titers of 1:160 or more were well above titers observed in the variety of acute and chronic conditions seen in a general hospital. Usually, atypical pneumonia patients with this high cold agglutinin titer had the more severe illnesses in which the diagnosis was well established on clinical grounds.

SUMMARY

1. The present report confirms and extends the list of conditions associated with the presence of cold agglutinins.

2. Cold agglutinins may be associated with exposure to a variety of noxious stimuli: Drugs, bacterial toxins, parasites, viruses, and blood letting. Common coughs and colds may be associated with cold agglutinins.

3. Several individuals with high cold agglutinin titers (1:160+) following one infection have been found to have rises in titer with subsequent infections. Cold agglutinins may persist after atypical pneumonia for 9 months, or cold agglutinins may appear and disappear without evident infection.

4. Atypical pneumonia is more often associated with the presence of serum cold agglutinins than other diseases studied in this report. A titer of 1:40 may be present in a variety of diseases. Among hospital patients in Boston, a titer of 1:160 is uncommon except in severe atypical pneumonia. When interpreted with other findings, the cold agglutinin test is useful in clinical diagnosis.

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STUDIES OF ACCOMMODATION OF NERVE IN PARATHYROID DEFICIENCY

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The properties of human nerve change with variations in the chemical content of the body fluids. When this change is pronounced, the altered condition of the nervous tissue is readily observed, as in tetany due to hypocalcemia or marked alkalosis. A quantitative measure of the condition of the nerves could be used to determine less obvious but perhaps significant variations in the functional characteristics of nerve. Such measurements would reveal the graded changes in nerve tissue associated with the gradual changes in the blood chemistry. The purpose of the present studies is to test the usefulness of a quantitative index of the changes in human nerve that accompany variations in the calcium ion concentration in the body fluids. The property of nerve employed is the rate of accomodation to an electrical current.

The process of accomodation can be described briefly as follows. The minimum constant current which just stimulates a nerve is called the rheobase. When the stimulating current rises slowly to its final value, the threshold strength of the current is greater than the rheobase. The slower the rate of rise of the current the higher is the threshold value. The property of a nerve by which it thus adjusts to the gradual development of the stimulating action of an electric current is called accomodation.

This property is markedly altered in excised nerves by variations in the concentration of ionized calcium in the fluids bathing the tissue (1). Furthermore, it can be readily measured in man as has been shown (1). An opportunity to study the variations in this property of human nerve in 5 cases of hypoparathyroidism was provided through the cooperation of the Medical Service of the Hospital of the University of Pennsylvania.[2] A test was being made of dihydrotachysterol, or A T 10, a drug used in the treatment of hypoparathyroidism. Complete calcium balance studies were made so that the quantitative measures of the functional state of the peripheral nerve could be compared with chemical changes.

Observations were made during a control period when large doses of calcium lactate and viosterol were given. The patients were then taken off all medication for periods of 7 to 12 days during which time they developed symptoms and signs of hypocalcemia. These manifestations consisted of numbness and tingling of the hands and feet, intermittent carpo-pedal spasm, and positive Chvostek and Trousseau signs. Upon administration of A T 10, their blood calcium level rose and symptoms of tetany disappeared. The functional state of the peripheral nerve was tested at intervals by determinations of the property of accomodation.

APPARATUS

The apparatus for the determination of accommodation of nerve was designed to deliver a current of rectangular wave form (*i.e.,* instantly rising) to be used for the determination of the rheobase, and exponentially increasing currents of various specified rates of rise for the determination of the threshold of the nerve to a series of slowly rising currents.

Approximate determination of the threshold of a nerve to currents passed through electrodes applied to the skin is readily achieved with practical values of current, if the cathode is placed near the nerve at a point where it lies close to the surface. An accurate determination of the threshold, however, would require a knowledge of the fraction of the current actually passing through the nerve fibers. This is practically impossible to determine, and for the present purpose it is unnecessary to do so. By a determination of the ratio between the threshold value of a slowly rising current and the rheobase a measure is obtained which is independent of the actual

[1] With the financial assistance of the Kirby-McCarthy Fund.

[2] These cases of post-thyroidectomy parathyroid deficiency were being studied by Dr. Edward Rose and Dr. F. W. Sunderman. A report of their clinical and chemical studies has been published. (Effect of dihydrotachysterol in treatment of parathyroid deficiency, Arch. Int. Med., 1939, 64, 217).

distribution of the current, provided this is the same for both determinations. The proportion of the total current passing through the nerve will be constant, if both the position of the electrodes and the impedance of the tissues remain unchanged between the determination of the rheobase and the threshold to an exponentially increasing current. The first of these conditions is obtained by making the two determinations in close succession. The second condition is largely satisfied by designing the stimulator so that the value of the current passed into the tissue is independent of the tissue and electrode resistances and their variations. Such a stimulator, used in the present study, has been developed and tested at the Johnson Foundation.

METHOD

One small electrode (cathode) was placed on the skin over the ulnar nerve at the elbow, and the other larger electrode made contact with the forearm.The least visible twitch of the first dorsal interosseus muscle or the least visible flexion of the distal phalanx of the thumb was used as an index of threshold stimulation of the nerve. By careful adjustment of the small electrode, one of these movements could be obtained as the only response to nerve stimulation. In any one patient, the same movement was used as the index of response throughout the experiments. The end-point was found by decreasing a suprathreshold stimulus until the least value of current which would produce a contraction was found. Slightly higher readings were obtained when the threshold was determined by gradually increasing a subthreshold current.

The threshold to an exponentially rising current was first obtained. Immediately thereafter, the rheobase was measured. The ratio (R) of these two values of current is a measure, expressed as a multiple of the rheobase, of the proportionate elevation of the threshold (degree of accomodation) to this particular slowly rising current. Currents of various rates of rise were used. In each experiment, therefore, the threshold to each of several slowly rising currents was measured and the degree of accommodation of the nerve was expressed in each case as a multiple of the rheobase. These data can be conveniently represented by plotting the values of R as a function of the time constant of the current rise (α). This representation is called the accommodation curve.

TABLE I

The values of the rheobase (I_0) and the threshold current (I) for slowly rising currents of different time constants of current increase (α) were obtained by stimulation of the ulnar nerve of a normal subject

Two successive trials were made for each value of α, using the least visible flexion of the distal phalanx of the thumb as the criterion for a threshold response. The values of the ratios ($R = I/I_0$) at each value of α are plotted to form the accomodation curve in Figure 1.

α	I_0 (Rheobase)	I	Ratio
milliseconds	*milliamperes*	*milliamperes*	
10	1.45 1.42	1.95 1.75	1.34 1.23
34	1.45 1.40	2.50 2.20	1.72 1.57
57	1.42 1.40	2.80 2.80	1.97 2.00
78	1.40 1.38	3.05 3.15	2.18 2.28
99	1.38 1.40	3.30 3.50	2.40 2.50
118	1.40 1.40	3.80 3.80	2.72 2.72

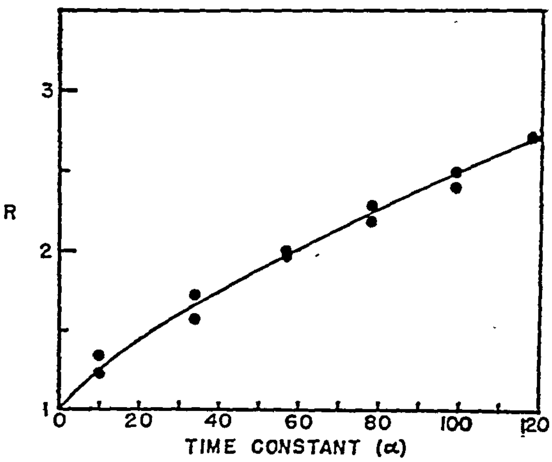

FIG. 1. A TYPICAL ACCOMMODATION CURVE FOR THE ULNAR NERVE OF A NORMAL HEALTHY MAN

R is the value of I/I_0 obtained from Table I; α is the time constant in milliseconds for the exponentially increasing currents employed for stimulation. The continuous line, represents the values of R calculated from equation 1 using a value of λ equal to 120 milliseconds.

OBSERVATIONS

Normal subjects. Measurements typical for the normal subject are given in Table I and are represented graphically in Figure 1. The percentage difference between the pairs of ratios (R) at each value of α illustrates the reproducibility of successive readings on the same subject. Repeated measurements on the same individual show little variation from day to day. Slight variations are occasionally found during a given day and may

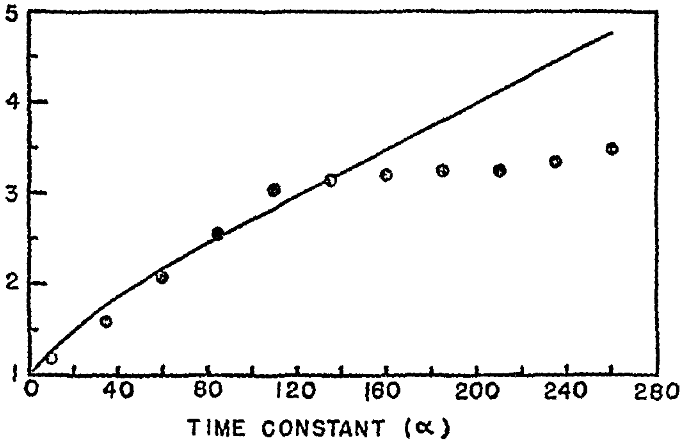

FIG. 2. THE RISE IN THRESHOLD TO SLOWLY RISING CURRENTS (ACCOMMODATION) AS MEASURED BY THE RATIO R DOES NOT CONTINUE TO INCREASE FOR VALUES OF α GREATER THAN ABOUT 140 MILLISECONDS

The points represent the average of 3 separate determinations of R at each value of α. The continuous line represents the calculated accomodation curve, using a value of λ equal to 95 milliseconds.

be related to fluctuations in the acid-base balance.[3] The results obtained from a series of 15 normal individuals are similar. In no determination did the ratio (R) at a value of α equal to 118 milliseconds exceed 3.5 or fall below 2.7.

The value of each ratio (Figure 1) depends upon the rate of rise of the stimulating current, as determined by α, and upon the rate of rise of the threshold of the nerve. The latter is regulated by a time constant λ which is characteristic of the nerve. The relation between R, α, and λ has been derived by Hill (2).

$$R = \frac{I}{I_0} = \left(\frac{\alpha}{\lambda}\right)^{\alpha/(\alpha-\lambda)} \tag{1}$$

The continuous line (Figure 1) represents the curve obtained from this equation using a value of λ equal to 120 milliseconds. The agreement between theory and experiment is good up to a value of α equal to 118 milliseconds.

When currents with slower rates of rise were used, no considerable further increase of the threshold was noted. The points in Figure 2 are the average of 3 measurements of R at each of the values of α indicated on the abscissae. The line represents the calculated values for a λ value equal to 95 milliseconds. There is considerable variation of the experimental values from the theoretical above values of α equal to 140 milliseconds. The "coefficient of accommodation" (λ) does not represent the experimental results except within a restricted range of values for α. Therefore, in the subsequent figures, either the complete experimental curve or the ratio (R) at 118 milliseconds is employed to show the changes in accommodation.

Subjects with parathyroid deficiency. The measurements of accommodation on these subjects during the control period were within the normal range. After removal of the calcium and viosterol therapy, the ratios (R) at each time constant (α) became *smaller than normal* on the first or second day, fluctuated at markedly subnormal values, and then rose rapidly after the administration of A T 10 to values above those observed during the "normal" control period.

Case 1. L. H., aged 42, thyroidectomy in 1923; symptoms of parathyroid deficiency for 15 years, relieved irregularly by parathormone, and calcium and viosterol therapy.

In Figure 3 is represented the response in this patient to withdrawal of calcium. This result is typical of all the patients with hypoparathyroidism who were tested.

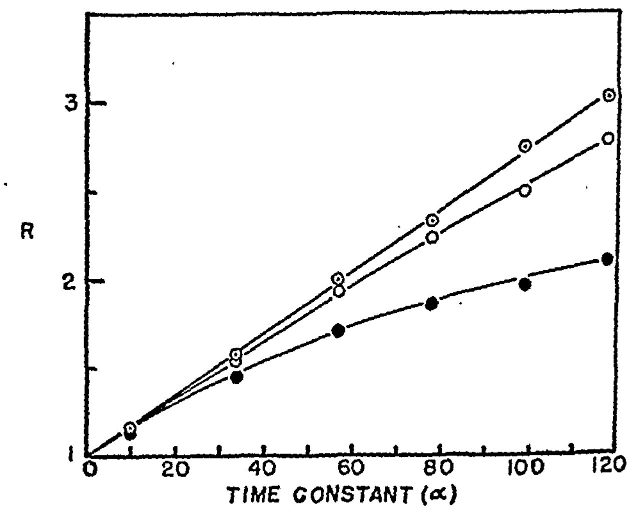

FIG. 3. CASE 1. THE CHANGE IN THE ACCOMMODATION CURVE OBSERVED WHEN THE LEVEL OF THE BLOOD SERUM CALCIUM IS FALLING AFTER DISCONTINUING CALCIUM THERAPY IN A PATIENT WITH PARATHYROID DEFICIENCY

Values of α are in milliseconds.

⊙ = ratios at the 6 values of α before therapy was discontinued (Nov. 14).

○ = ratios observed on the first day without calcium therapy (Nov. 15).

● = ratios observed on the fourth day without calcium therapy (Nov. 18).

[3] In latent tetany, these variations become of enough significance to be misleading unless readings are taken at the same time of the day, or several times during the same day.

Calcium lactate and viosterol had been given up to and including November 14th. On November 15th, no calcium was given, and the values of R for each of the slowly rising currents were found to be less than the control values. At this time, there were no clinical symptoms or signs of increase in nerve irritability. The measurements on November 18th, the fourth day without therapy, revealed a marked decrease in the rate of accomodation as indicated by values of R much lower than those obtained during the control period. By this time, clinical examination revealed early signs of increased nerve irritability.

In Figure 4, the response to administration of A T 10 is charted. No therapy was given from November 15th to 26th, inclusive. The values of the ratios measured on November 26th remained markedly reduced. On the morning of November 27th, A T 10 was administered, and 2½ hours later, there was an increase in each of the values of R, indicating an increased ability of the nerve to accommodate. At this time, there was no clinical evidence of improvement. Further records taken on the third and sixth days of A T 10 administration are shown, each illustrating additional improvement in the physiological state of the nerve as determined by this method. The record of December 1st indicates a degree of accommodation even greater than that in the normal subjects. Smaller doses of A T 10 were given thereafter, and subsequent observations showed a return of the nerve processes to the normal state.

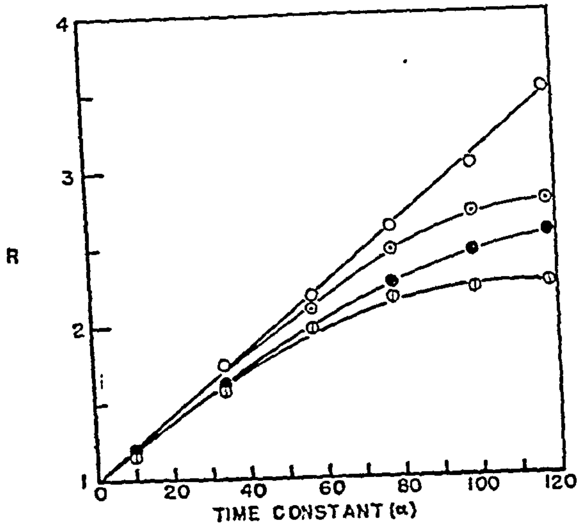

FIG. 4. CASE 1. THE CHANGE IN THE ACCOMMODATION CURVE OBSERVED DURING THE RISE IN THE LEVEL OF SERUM CALCIUM AFTER ADMINISTRATION OF DIHYDROTACHYSTEROL (A T 10)

Values of α in milliseconds.

ⓘ = Values of R on Nov. 26th—twelfth day without therapy.

● = Values of R on Nov. 27th—2½ hours after administration of A T 10.

⊙ = Values of R on Nov. 29th—the third day of A T 10 administration.

○ = Values of R on Dec. 1st—the fifth day of A T 10 administration.

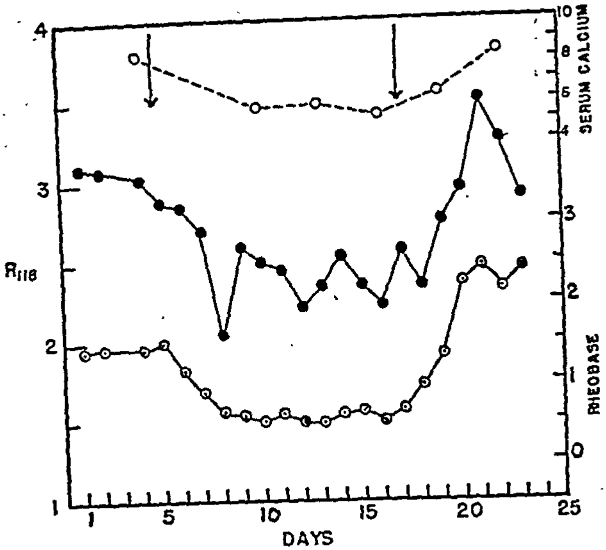

FIG. 5. CASE 1. CHART SHOWING RELATIONSHIP OF SERUM CALCIUM VALUES, RHEOBASE VALUES, AND THE VALUES OF THE RATE OF ACCOMODATION (AS REPRESENTED BY THE VALUE OF THE RATIO WHEN α IS EQUAL TO 118 MILLISECONDS)

The first arrow indicates the time of withdrawal of maintenance dosage of calcium and viosterol. The second arrow indicates the beginning of administration of A T 10.

The relation of the ratios to the level of the blood serum calcium is shown in Figure 5. The degree of accomodation is represented by the value of the ratio when α is equal to 118 milliseconds. The total serum calcium is measured in milligrams per cent.[4] The changes in the degree of accommodation upon withdrawal of calcium and upon administration of A T 10 parallel the changes in the blood calcium. Included in Figure 5 are the average values of the rheobase measured during these experiments. The change in the stability of the nerves, indicated by these threshold values of direct current, also parallels the change in blood calcium.

Case 2. M. H., aged 30 years; thyroidectomy July 1935; hypoparathyroid tetany appeared on the following day and was subsequently forestalled with moderate success for 2½ years by the use of calcium, viosterol, and parathyroid hormone. Studies were started February 10, 1938; therapy discontinued after February 13th; A T 10 given, starting February 20th.

The withdrawal of calcium and viosterol was followed by a marked decrease in the rate of accommodation. After administration of A T 10, restoration of R to values above the normal was observed. The changes in

[4] The ratio of diffusible calcium to total calcium was approximately constant for each subject. The diffusable calcium was about equal to the calculated calcium ion concentration. (See paper by E. Rose and F. W. Sunderman for details.)

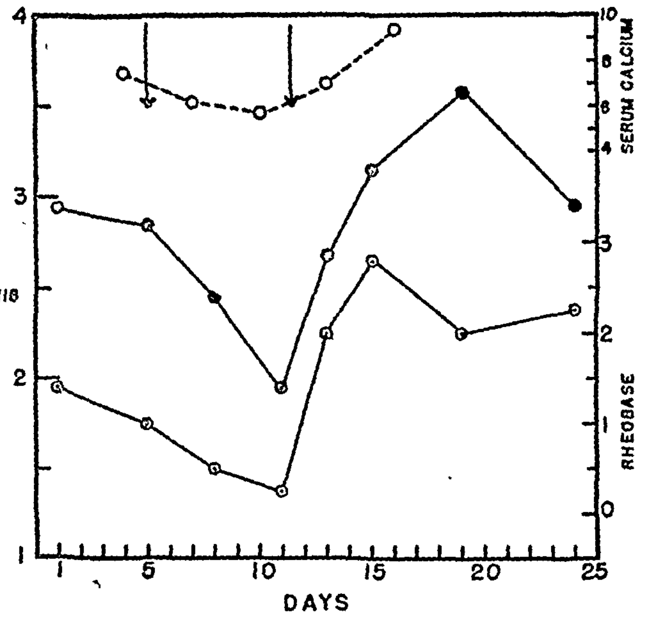

Fig. 6. Case 2. Illustrating the Relationship of Serum Calcium, Rheobase, and Ratios at 118 Milliseconds, in Another Case of Parathyroid Deficiency

the blood calcium level were similar to those in the first case. These changes are illustrated in Figure 6.

Case 3. J. J., aged 38 years; hypoparathyroid symptoms for 10 years following thyroidectomy, partially relieved by calcium and viosterol. Studied in September, 1937; calcium withdrawn September 14th; A T 10 given September 21st.

When the calcium was discontinued, there was a very marked decrease in the rate of accomodation. After administration of A T 10, the rate of accomodation increased as usual from an abnormally low figure to a value at the lower limits of the normal range. The changes in the level of blood calcium during this period were similar to those previously noted.

Case 4. H. E., aged 39 years; post-thyroidectomy hypoparathyroidism had been present for 20 years in this patient. Symptoms varied but were usually relieved by the administration of calcium, although the blood calcium remained persistently low (6 to 8 mgm. per cent). Studied December 1937; calcium withdrawn December 13th; A T 10 given December 20th.

At the end of the control period, the values of the ratio at each time constant were normal, yet at this time the blood serum calcium value was quite low (7.9 mgm. per cent). After withdrawal of therapy, the decrease in the rate of accomodation was moderate and not as abrupt as in the other cases. The blood calcium level also fell but moderately (to 6.8 mgm. per cent on the sixth day.) After administration of A T 10, there was a marked rise in the values for the rate of accommodation, and the blood calcium.

Case 5. M. R., aged 29; subtotal thyroidectomy, September 1937; subacute symptoms of hypothyroidism and hypoparathyroidism after operation. Admitted to the hospital in January 1938 for study. This patient had not been receiving substitution therapy. Therefore, the initial observations were made at a time when the blood calcium was abnormally low.

During this period without therapy, the rate of accomodation was also found to be abnormally low. After administration of A T 10, the rate of accommodation and the level of serum calcium increased in parallel fashion. The rheobase also rose markedly.

Several evidences of functional changes in the central nervous system are worthy of mention. In all cases, the tendon reflexes were hypoactive. Three cases complained of being unduly apprehensive and fearful, particularly during the periods when the blood calcium was low. One noted impairment of recent memory during the hypocalcemic periods.

DISCUSSION

The studies of subjects with parathyroid deficiency indicate that characteristic alterations of nerve function accompany changes in the blood calcium level and that these can be expressed quantitatively by measurement of the degree of accommodation of the nerve.

It is to be noted, however, that a given accommodation curve cannot be considered as characteristic of a given value of the serum calcium. A normal accomodation curve may be obtained when the blood calcium is relatively low, if this low value has been present for a considerable time. Presumably, a gradual physiological adjustment to this low level may take place. In contrast to such long-time adjustments, every rise and fall of the level of the blood calcium taking place within a period of several days is accompanied by corresponding changes in the rate of accomodation. These changes are measurable before clinical signs and symptoms of the altered blood chemistry are apparent.

The critical value of the threshold stimulus is clearly defined. It was noted that, as successively more slowly rising currents were used, the muscular response became progressively slower in onset and continued in partial tetanus during the flow of the current. A slow contraction rather than a clear-cut twitch is then observed. In the subjects with low blood calcium and slower accommodation, the slow onset and tetanic nature of the contraction occurred with definitely lower values of current strength and with more rapidly rising currents. This is another indication of the reduced ability of the nerves in such subjects to accommodate to the electrical stimuli.

One theory (2) predicts an accommodation curve which gradually approaches a limiting straight line of finite slope. The observed curves follow, in general, the predicted course up to values of α equal to 118 milliseconds. Beyond this point, the curve becomes nearly horizontal (Figure 2). This form of curve has also been found in an earlier investigation (3). It would seem that not only does accommodation proceed at a certain rate, but also that the threshold reaches a certain value beyond which it rises but slowly or not at all. For these reasons, as was previously noted, the index of the rate of accomodation used was the graphic representation of the entire set of ratios. By the comparison of a series of determinations, it was found that the value of the ratio obtained when α equaled 118 milliseconds represented faithfully the variations shown by the total curve (Figures 5 and 6). Using either of these indices, it has been found that normal values fall within a limited range and that the amount of variation in repeated determinations is about $\pm$ 5 per cent of the average value.

The well-known alterations in the threshold of the nerve to abruptly rising currents (rheobase) associated with a falling or rising blood calcium level were observed in these studies. This change is an additional indication of the altered physiological state of the nerve. The values of the observed rheobase, however, were found to be subject to considerable variation, depending upon the position of the stimulating electrode and probably the thickness of the skin. In contrast, deliberate or accidental changes in the apparent rheobase, within certain limits, failed to alter the observed values of R provided the two determinations of threshold used to calculate R were made under comparable conditions.

It is to be noted that the change in the rheobase due to alteration in the blood calcium does not account for the changes in accommodation. A decrease in the rheobase, when the blood calcium decreases, would tend to increase the value of R, whereas, it is observed that R also decreases under such conditions.

These observations show that a quantitative objective measure of a physiological change in the nerve tissue of patients with hypoparathyroid disease can be obtained. It is thus possible accurately to compare the changes in blood calcium with the altered functional condition of the nerve tissue. This measure of the condition of the nerves was just as adequate as an index of the response to A T 10 of patients with parathyroid deficiency as was the measure of the concentration of blood calcium. The procedure could be employed to determine the amount of A T 10 (or other medication) required to maintain normal the properties of nerve tissue.

SUMMARY

1. A measure of the property of accommodation in human peripheral nerve can be readily obtained with consistent results.

2. The method used has the advantage of being relatively independent of certain variable factors which may impair the accuracy of quantitative measurements of peripheral nerve irritability made through the intact skin.

3. In agreement with previous observations (3), the accommodation curve for human nerve is found to be terminated by a horizontal portion which is probably associated with repetitive firing in the nerve fibers.

4. In 5 cases of hypoparathyroidism, the functional state of the peripheral nerve as indicated by measures of the property of accommodation was found to vary characteristically with changes in the calcium ion concentration in the blood.

5. This method of studying this disease is useful whenever a detailed quantitative measure of the changes in the state of the nerve tissue is desired. The quantitative measure of the functional change makes possible a relatively accurate comparison of the degree of change in the nerve tissue with the degree of chemical change in the blood and tissue fluids.

The authors wish to acknowledge their indebtedness to Professor D. W. Bronk for suggesting their collaboration in this research.

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ON THE DETERMINATION OF ARTERIAL OXYGEN SATURATIONS FROM SAMPLES OF "CAPILLARY" BLOOD [1]

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The attack upon many problems in respiratory ysiology requires the determination of the ygen saturation of the arterial blood. These erminations in the past have been limited by difficulties attending repeated arterial puncture man. It has long been believed that the oxygen itent of cutaneous finger blood approximates it of arterial blood. Indirect evidence in suprt of this belief was first furnished when it was nonstrated that the oxygen saturation of blood tained from a vein on the dorsum of the heated nd equaled that of blood from the brachial ery (1).

Later it was shown (2) that the oxygen saturan of blood obtained from an incision of the ger tip was equivalent to that of arterial blood. iis relationship offered the possibility of estiiting the arterial oxygen saturation by analysis cutaneous blood, but the difficulty in obtaining nples of adequate volume prevented the developint of this method. A step toward the solution this problem has been furnished by the recent velopment of a microgasometric method for the alysis of blood gases (3). This method makes ssible accurate determinations on small samples blood (0.04 ml.).[3] However, the collection finger samples large enough for duplicate dermінations of both oxygen content and capacity ay require massage which results in dilution of the sample with tissue fluid. This difficulty has been obviated by the use of the ear lobe as a source of "capillary" blood.

This communication reports (*1*) a simple method for determining the oxygen saturation of samples of cutaneous blood from the heated ear; and (*2*) the experimental demonstration that the oxygen saturation of cutaneous blood obtained from the heated ear corresponds, within the limits of analytical accuracy, to the oxygen saturation of blood obtained simultaneously from the brachial artery.

METHODS

The collection of blood samples from the ear lobe necessitates a brief contact of blood with the ambient air. Despite the virtually instantaneous uptake of oxygen by solutions of hemoglobin, it was discovered that a drop of blood may be allowed brief contact with air without effecting a measurable change in oxygen content. This may be due to the negligible number of erythrocytes which are exposed to the gas phase. In order to estimate the effect of such contact with air, the following experiment was performed. The oxygen content of a sample of oxalated venous blood was determined both by the Roughton-Scholander and the Van Slyke-Neill methods. Another portion of the same sample was dropped slowly through air into the collecting funnel and syringe, employed in the method described below, and the oxygen content was determined by the microgasometric method. The results of these analyses follow:

Oxygen content in volumes per cent

Anaerobic sample		Mean
Van Slyke-Neill	5.9, 6.2	6.0_5
Roughton-Scholander	5.9_5, 5.9_5	5.9_5
After transfer dropwise through air		
Roughton-Scholander	5.8_5, 5.8	5.8_5

It is apparent that no significant change in oxygen content had occurred as a result of the transfer of blood dropwise through air.

The ear lobe provides an excellent source of "capillary" blood because a deep puncture is virtually painless. Furthermore, full vasodilatation to provide rapid blood flow can be effected easily by the local application of

[1] The opinions or assertions contained herein are the ivate ones of the writers and are not to be construed official or reflecting the views of the Navy Department the naval service at large.

[2] Lieutenant (MC) USNR

[3] The accuracy of the microgasometric method has en checked in this laboratory by comparison of ialyses performed on the same samples of blood by e Roughton-Scholander method and by the Van Slykeeill manometric method. The correlation has been und to be within 0.3 volumes per cent. For duplicate ialyses by the Roughton-Scholander method, only checks ithin 0.3 volumes per cent are acceptable.

heat. The ear is warmed best by radiant heat. The oro-nasal rubber mask furnished for basal metabolism determinations may be converted readily for use as a heater. The inner surface is covered with aluminum paint or any suitable reflecting material and the inlet is modified to hold a small electric light bulb as a source of heat. The mask is then fitted over the ear. Within a few minutes this heater produces full local vasodilatation despite low ambient temperatures.

The ear lobe is punctured with a sharp pointed knife. The puncture should be deeper than the stab used for routine hematological samples and should produce a steady dropping of blood *without any massage*. The blood is collected directly from the ear into a 2 ml. Luer syringe by means of a small glass funnel (Figure 1). Before use, the syringe is rinsed with a 15 per cent

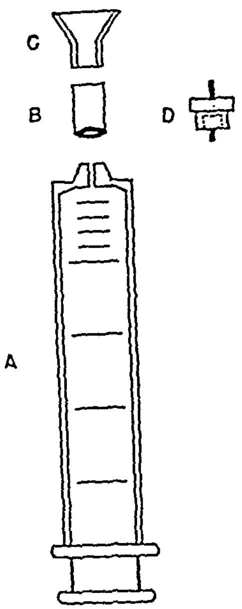

FIG. 1. A. 2 ML. LUER SYRINGE WITH SHORTENED TIP TO REDUCE DEAD SPACE. B. RUBBER CONNECTING TUBE. C. SMALL GLASS COLLECTING FUNNEL. D. RUBBER STOPPER WITH WIRE PLUG.

solution of potassium oxalate; the plunger of the syringe is advanced fully and the remaining solution prevents coagulation and obliterates the dead space. As the drops of blood fall into the funnel, they are drawn rapidly into the syringe without admixture of any air bubbles. A sample of 0.4 to 0.5 ml. will furnish ample blood to perform the microgasometric analyses in triplicate for both oxygen content and capacity. The funnel and connecting tube are then removed from the syringe and replaced by a rubber stopper with a small hole which is occluded by a removable fine wire. As soon as the syringe is thus sealed it is rotated until all analyses are completed. The Roughton-Scholander sampling pipette may be introduced through the hole in the rubber stopper and aliquots of blood withdrawn under anaerobic conditions. After completion of the analyses for oxygen content, the stopper is opened and 1.5 ml. of air is drawn into the syringe; rotation is resumed. This air is expelled and replaced every 5 minutes for 3 changes. At the end of 20 minutes, the blood has become completely oxygenated and the microgasometric analyses for oxygen capacity may be performed.

RESULTS

Oxygen saturations in peripheral and arterial blood have been compared at various levels of oxygen saturation and of ambient temperature by means of appropriate oxygen-nitrogen mixtures administered at sea level and by exposure to simulated pressure-altitudes in the chilled low pressure chamber. In these studies, the samples of arterial blood were drawn at the same time that the cutaneous samples were obtained from the heated ear. This was accomplished by introducing an indwelling needle with blunt stylet into the brachial artery before exposure to low oxygen pressure was begun. The results of these experiments are presented in the accompanying table.

TABLE I

Simultaneous "capillary" and arterial oxygen saturations **

Subject	Altitude	Breathing	Temperature	Percentage saturation		
	feet	*per cent O_2*	°C.	*Ear lobe*	*Artery*	*Difference*
Fu	Sea level	12.0	24	84.3	87.0	−2.7
Ri	Sea level	13.0	24	82.0	84.0	−2.0
Fu	Sea level	13.0	24	86.9	87.3	−0.4
Li	Sea level	13.0	24	84.0	86.2	−2.2
Ri	14,000	20.94	22	75.7	76.2	−0.5
Li	14,000	20.94	22	80.5	80.6	−0.1
Ri	14,000	20.94	−20	77.5	74.4	+3.1
Li	25,000	"100.0"	−20	96.8***	96.4***	+0.4
Du	Sea level	20.94	24	97.7	98.3	−0.4
Po	Sea level	20.94	24	96.7	96.9	−0.2
Li	Sea level	20.94	24	94.3	94.8	−0.5
Ro	Sea level	20.94	24	96.8		
Ri	Sea level	20.94	24	95.6		
Li	Sea level	"100.0"	24	102.2***		
Ro	25,000	"100.0"	22	102.0***		

** These data represent a series of consecutive experiments made after the preliminary trials during the evolution of the method.

*** Not recalculated for dissolved oxygen due to higher partial pressures.

The experiments presented indicate that there is no significant difference between the oxygen saturations of arterial blood and of cutaneous blood obtained from the heated ear. The variations in the samples obtained at the same time are no greater than might occur in duplicate determinations in which the range of accuracy of the analysis is 0.3 volumes per cent.

The volume of the wet anti-coagulant employed in this method is sufficiently large with respect to the size of the blood sample to preclude estimation of the absolute oxygen contents and capacities. However, the dilution factor for content and capacity is the same in any one sample so that the determination of saturation is accurate. Absolute contents might be determined, if required, by weighing the dry syringe, weighing again after the addition of the anti-coagulant, and finally after the introduction of the blood sample, in order to calculate the actual dilution. The use of a dry anti-coagulant and a mineral oil seal has not proved feasible with these small samples of blood.

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A NEW METHOD FOR STUDYING BREATHING, WITH OBSERVATIONS UPON NORMAL AND ABNORMAL SUBJECTS

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METHOD

The apparatus described in this article is a simple device that will record the changes in the rate of air flow with time during each respiration and without the lag and inertia of other methods. The instrument designed for this purpose consists of a fine platinum wire 200 mm. long and 0.01 mm. (10 microns) in diameter, suspended across the diameter of a 31.6 mm. tube. One end of the wire is attached to a fixed point and the other end to a fine spring. The displacement of the wire when air flows through the instrument is recorded photographically by a moving paper camera. The deflection of the wire is essentially linear with air flow, and the inertia, lag, and frequency of vibration do not interfere with respiratory measurements. Unlike other instruments devised for this purpose, this apparatus does not introduce significant resistance to air flow and, therefore, does not affect normal respiration.

The complete measuring apparatus is shown in Figure 1. The inspiratory instrument, with a bell-mouth to prevent turbulent air currents and decrease resistance, is shown at A. The expiratory instrument, with heating bath for preventing condensation in the line, is shown at B. The recording wires of these two instruments are shown schematically in the lower part of the figure. The valves for producing directional air flows through the two instruments are shown at C. A large spirometer with slide valves for shifting from the recording instrument to the spirometer for collection of expired air is also shown in Figure 1. A small half mask with minimal dead space is provided with a Heidbrink pneumatic cushion to ensure an air-tight seal to the subject's face.

In a recent paper (1), a description was given of the details of construction of this apparatus as adapted to measurements of air flow during inspiration. Since that publication, the device has been modified to record both inspiratory and expiratory air flows. The changes made in the original inspiratory instrument to record expiratory air flows are as follows. To eliminate the effect of the turbulence of the expired air, a fine mesh wire screen (150 mesh Monel wire) is located 5 mm. from the recording wire, and to prevent condensation from expired air in the instrument, a heating bath and a straight section of tubing are inserted in the line before the wire screen. To compensate for the loss in cross sectional area of the tube caused by the wire screen and to reduce the resistance to an amount equal to that of the inspiratory instrument, it was necessary to increase the diameter of the tube of the expiratory instrument from 31.6 to 47.8 mm. Lastly, to prevent extraneous oscillations of the expiratory wire, the lower leg of the channel housing the wire is partially filled with kerosene which acts as a viscous damper. The évasé, a megaphone-shaped outlet shown on the end of the expiratory instrument, also decreases the expiratory resistance. In addition to the above changes, a new valve has been devised, which is indicated on the mask at C in Figure 1. This valve has a resistance of less than 1 mm. of water at an air flow of 100 liters per minute and has a negligible opening pressure. A detailed description of the valve will appear elsewhere. The over-all resistance of the measuring apparatus to either inspiration or expiration is less than 2 mm. of water at 100 liters per minute. This includes the resistance of the spirometer, which is equipped with a well-counterbalanced aluminum bell and a connecting tube of large diameter (50.8 mm.).

DESCRIPTION OF TRACINGS

Typical records for a normal subject (L. S., male, 29 years) are shown in Figure 2. In the upper half of each record is the curve of expiratory air flow and in the lower half, the curve of inspiratory air flow. The time in intervals of one-fifth of a second is indicated by the horizontal line at the top of each record. The curves are read from left to right. The ordinates or vertical movements of the curves on the record represent the volumes of instantaneous air flows at the times indicated by the abscissae or horizontal movements of the time line.

The upper record (I) in Figure 2 represents tracings obtained with a normal subject without any resistance in the line. The curve for normal inspiratory air flow starts at A, has a fairly sharp change to a maximum air flow (as indicated by the steep downward limb of the curve of the inspiratory cycle), then tapers off in flow, and rises

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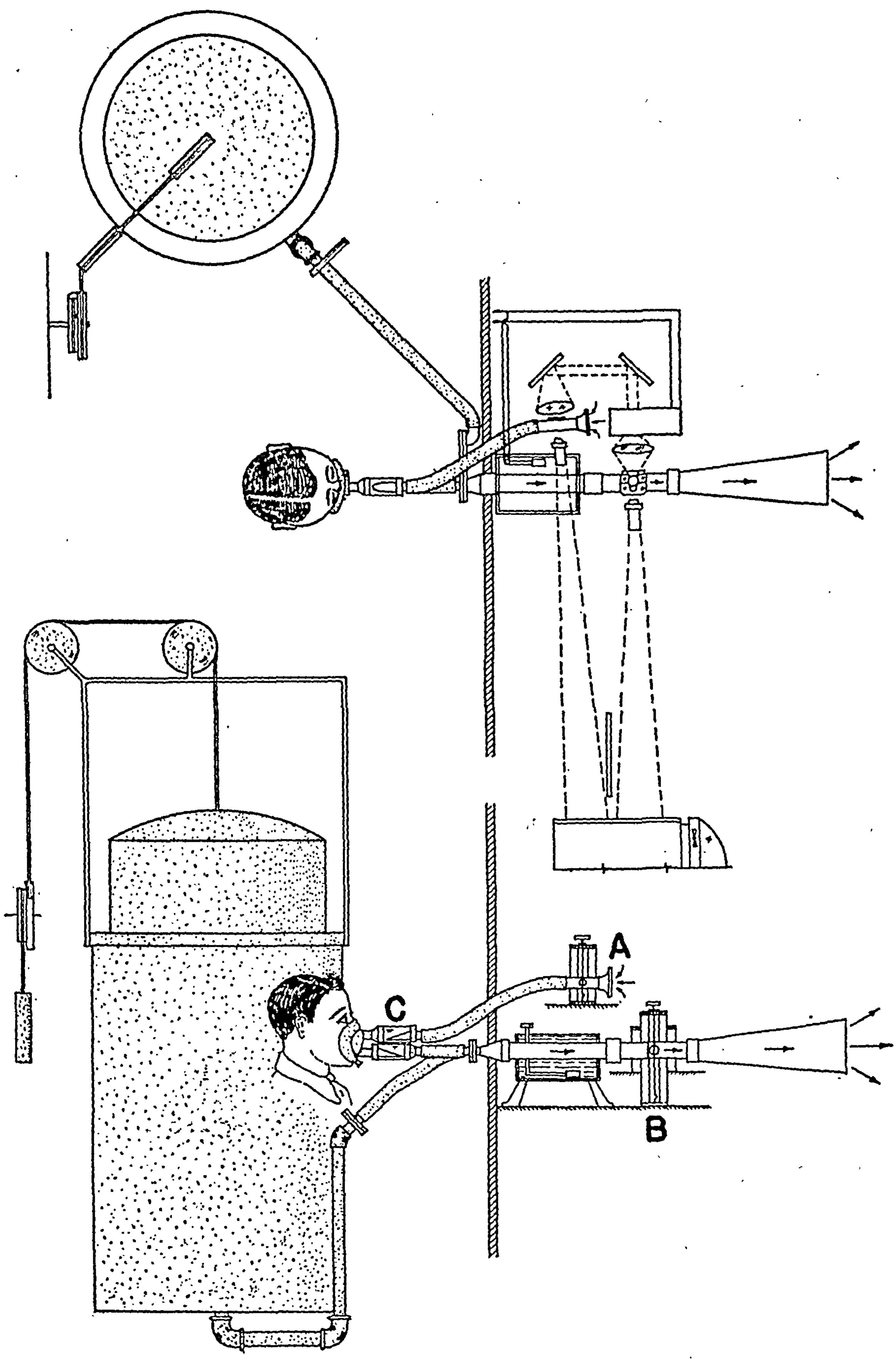

FIG. 1. DIAGRAM OF APPARATUS FOR MAKING A PNEUMOTACHOGRAM AND OTHER MEASUREMENTS ON BREATHIN
Description in text.

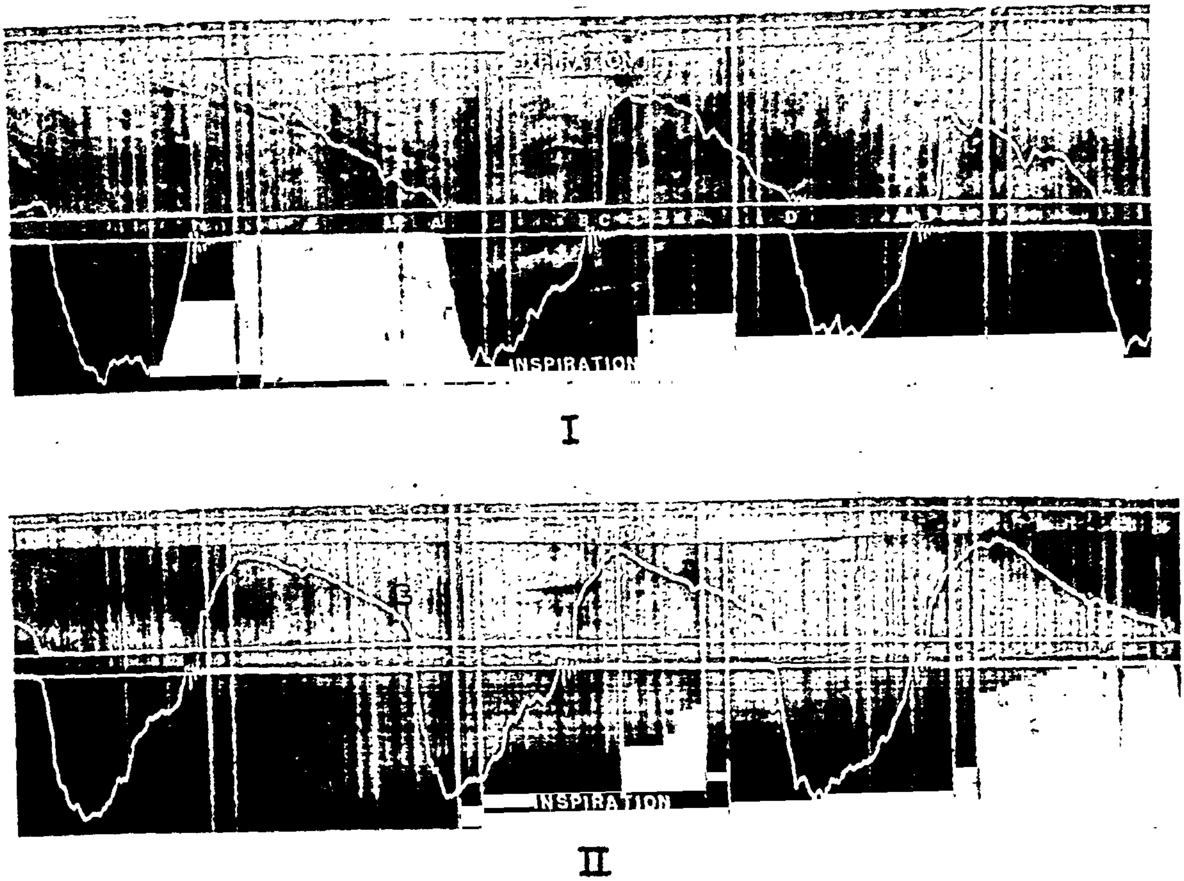

Fig. 2. Typical Tracings Recording the Respiratory Air Flow of a Normal 29-year old Man, Without Resistance (upper record, I) and With Resistance (lower record, II) to Expiration

Inspiration begins at A and continues to B. The maximum rate of inspiratory air movement occurs abruptly, as indicated by the downward limb of the curve, and the inspiratory flow falls off to zero during the gradual upward limb of the curve ending at B. Expiration begins at C with a rapid movement of air, and the flow falls off to zero at D. In the lower record, the same subject breathed against resistance to expiration of 0.9 mm. of water per liter of air flow per minute. In all records, the areas bounded by the zero line and an inspiratory curve or the zero line and an expiratory curve from start to end represent the volume of air per breath or tidal volume.

towards the base-line until it reaches zero flow, when it crosses the horizontal line for zero flow at B. The curve for normal expiratory air flow begins at C, its rising limb rapidly reaches a height indicating maximum expiratory flow, and the curve then falls off gradually to zero flow at D. These tracings should not be confused with the ordinary spirogram, in which upward movement represents inspiration and downward movement expiration. Each pair of curves in these records is a complete record of the pattern of air flow during each phase of respiration. The area bounded by the zero line and the curve of inspiration between A and B or the area bounded by the zero line and the curve of expiration between C and D represents the volume of air per breath or tidal volume. By adjustment of the tension of the wires, both of the recording wires are set to have approximately the same sensitivity, at a constant air flow, which is provided by a calibrated rotameter or flow meter. The slight differences between the areas bounded by the inspiratory and the expiratory curves and the zero lines in Figure 2 and in succeeding figures are ascribable to slight differences in the adjustments of sensitivity of the inspiratory and the expiratory instruments. The wires are calibrated individually at a constant air flow, for critical analysis of the tracings.

The individual characteristics of breathing resulting from jerky muscular movements cause the variations in contour of the curves throughout the tracing. In the background, on the tracing lines, the free vibrations of the measuring wire at a much higher frequency of air flow are apparent.

The lower record (II) in Figure 2 was obtained on the same subject when a resistance had been

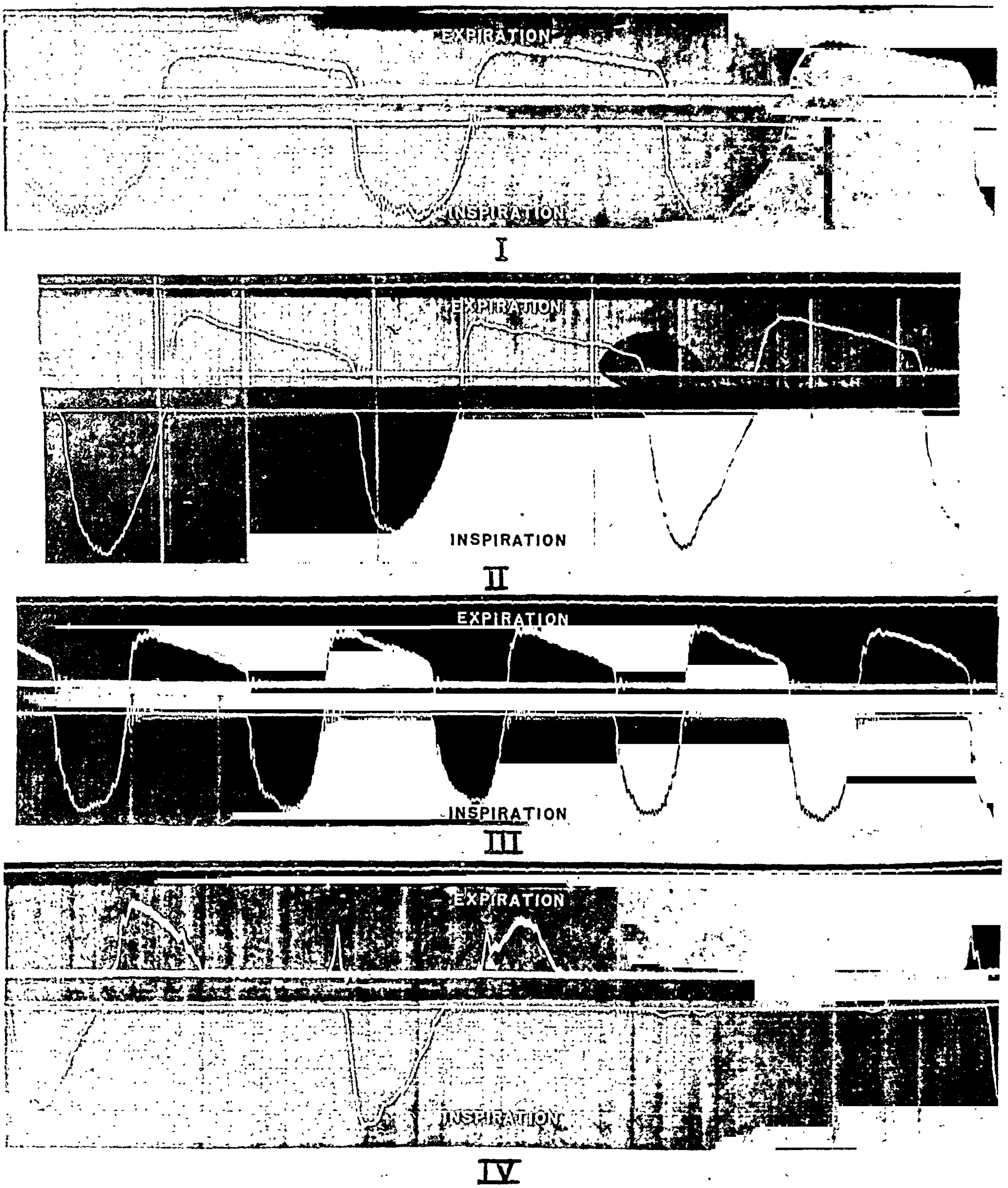

Fig. 3. Pneumotachograms from 4 Patients Under Observation in the Allergy Clinic of the Massachusetts General Hospital in the Service of Dr. Francis M. Rackemann

Descriptions in text.

inserted in the expiratory line. This resistance was made by a glass filter cloth, which was held between two flanged funnels. The funnels were heated to prevent any adsorption of moisture and change of resistance. The resistance of this filter cloth arrangement increases linearly with increasing air flow and has a value of 0.9 mm. of water per liter of air flow per minute. With this amount of expiratory resistance, the flow of expiratory air is maintained at a measurable volume because of the forced expiration until the end of the expiratory phase or effort, when the flow drops off suddenly. In this type of expiration, the gradual approach to zero flow is absent, the expiratory tracing is much smoother and indicates a sudden fall of flow at the end of expiration rather than the gradual return to zero flow shown by the tracing for the same subject without the resistance in line.

The viscous damping of the expiratory instrument produces a very slight lag in the return of the curves of expiratory air flow to the zero line, if this occurs suddenly, that is, in less than 0.04 second.

OBSERVATIONS UPON PATIENTS

As experience with the method accumulated and the sensitivity of the new spirograms (pneumotachograms) for portraying characteristics of breathing entirely unrecognizable in the usual curves became apparent, it was obviously worth while to see what the method would show when used for examination of patients with abnormal breathing. Accordingly, Dr. Francis M. Rackemann was asked to send 8 patients to the laboratory. These individuals had long records of observation in the wards and in the Allergy Clinic of the Massachusetts General Hospital. All were equal to limited physical activities, and no one was in the least annoyed by the procedures involved in making the records. Indeed, the cooperation of subjects in this type of test is less exacting than that required in determination of the basal metabolism. The subject sits comfortably in an armchair. The face mask with valves attached is applied, and natural breathing continues. The recording apparatus is on the other side of a partition, and the patient has no knowledge of when records are being made or in what ways the act of breathing is being hindered or assisted, if such changes are used to display or magnify peculiarities in respiration.

The compelling nature of other work has prevented us from the extensive clinical studies that the method merits. Our purpose in this paper is to show some of the results obtained with the new technic and to make this technic available for clinical employment by those interested in the problems of abnormal breathing. Accordingly, in Figures 3 and 4 are presented single records

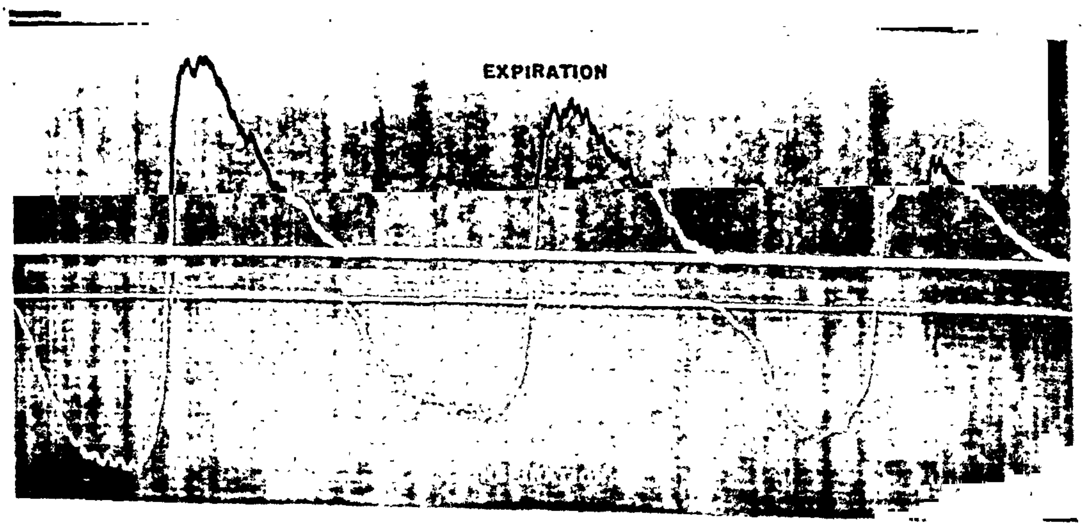

FIG. 4. PNEUMOTACHOGRAM FROM A FIFTH PATIENT FROM THE ALLERGY CLINIC
Description in text.

TABLE I

Results of pneumotachographic observations on a normal man and on patients with respiratory abnormalities

Condition and subject	Respiration rate	Minute volume	Respiratory quotient	Mean maximum air flow	
				Inspiration	Expiration
	per minute	*liters*		*liters per minute*	
Normal (L. S.)					
No resistance to expiration	15.8	8.3	0.77	25	23
Resistance to expiration	15.3	7.7	0.79	31	20
Patients with respiratory abnormalities					
J. P.	15.8	11.6	0.97	38	25
W. E. D.	16.7	9.3	0.91	53	28
W. F. L.	22.0	9.2	0.88	37	27
J. M.	10.2	5.4	0.80	40	30
S. M.	12.0	7.9	0.75	51	51

from 5 patients, and in Table I are summarized the results of the observations on these patients and likewise those on the normal subject previously mentioned.

Patient 1, J. P., male, 46 years (pneumotachogram I, Figure 3). Since the age of 38, the patient has had asthma. His symptoms are related to exertion. In pneumotachogram I, inspiration and expiration are smooth and regular in character. Expiratory movement of air is in the nature of a slow push and ceases entirely a little before the cycle of expiration is finished. When expiration ceases, the flow drops quickly to zero, as was the case in the normal subject when resistance was placed in the expiratory line (Figure 2, lower half, II). The rounded character of the inspiratory curve indicates some degree of resistance to inspiration.

Patient 2, W. E. D., male 50 years (pneumotachogram II, Figure 3). At the age of 40, the patient began to have dyspnea on exertion, and now is a typical instance of emphysema accompanied by asthmatic attacks. The similarity of his record (pneumotachogram II) to that of the first patient requires no comment.

Patient 3, W. F. L., male, 19 years (pneumotachogram III, Figure 3). The patient has had asthma for 17 years. The tracing is again characteristic of breathing obstructed at expiration and somewhat so at inspiration.

Patient 4, J. M., male, 63 years (pneumotachogram IV, Figure 3). Asthma began at the age of 45 in isolated attacks but was not bad until his 50th year, when he was admitted to the hospital for treatment. This is an instance of extreme emphysema and asthma. When pneumotachogram IV was obtained, the patient was breathing irregularly and was obviously ill but did not object to wearing the face mask and cooperated well in the observation. His breathing consisted of complete respiratory cycles with trifling inspiratory and expiratory efforts in between. Most of these would not be noted by any other means of observation. The failure of this patient to provide the type of expiratory curve characteristic of the previous patients is apparently ascribable to loss of ability to continue to force air out of the lungs. However, there is a final expiratory effort or blow just before inspiration begins, which causes the small expiratory "spike" before the inspiratory curve.

Patient 5, S. M., male, 44 years (pneumotachogram in Figure 4). This man has had hay fever since the age of 30. He is a pessimistic individual who complains of more symptoms than seem to be present. The curves in Figure 4 are of unequal extent but are repetitive in configuration. The expiratory curve does not have the contour characteristic of the curves of the asthmatic patients mentioned above. However, the inspiratory phase indicates that some inspiratory resistance is present. The inspiratory curves are similar to those of patient 3.

The data in Table I were obtained at the time of photographing the records shown in Figures 2, 3, and 4. The maximum flows and respiration rates were calculated directly from the pneumotachograms.

DISCUSSION

In addition to the features of the pneumotachograms discussed above, another point of interest noted thus far is the tendency of the individual to maintain a uniform configuration of inspiratory and expiratory curves. This is one of the characteristics of the pneumotachogram which should make the technic profitable in following the condition of patients during the progress of or recovery from a respiratory disease, for the repeated examination of young men in training, and for the examination of children during growth. The absolute objectivity of the method and the ease with which the subject is kept unconscious of his moment of participation in the test may be of use in psychologic and psychiatric studies. The apparatus can be constructed by a good mechanic furnished with the

directions available in the paper by Lee and Silverman (1).

SUMMARY

A new pneumotachographic device is described for making a graphic record of the velocity of air movement during inspiration and expiration. The inspiratory and the expiratory air currents cause the deflections of fine wires, deflections that are recorded photographically by a moving paper camera. Typical illustrations of the resulting curves are presented, showing the results on one normal individual and on 5 patients with respiratory difficulties. The total minute volume and the instantaneous air flow can be obtained from the records made by the instrument.

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RENAL EXCRETION OF SULFAMERAZINE[1]

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Certain advantages might accrue to the use of sulfonamides such as sulfamerazine (2-sulfonamido 4-methyl pyrimidine) that, having essentially the same antibacterial activity as others in common use, are characterized by low rates of renal excretion (1 to 6). These advantages include lower and less frequent dosage and, perhaps, some reduction of the renal hazard incidental to the precipitation of material in the kidneys and urinary tract. It was because of these considerations that it was deemed advisable to study some of the factors concerned with the excretion of sulfamerazine. Observations were also made on the excretion of the N^4—acetyl derivative of sulfamerazine and of sulfadiazine (2-sulfanilamido pyrimidine) for comparative purposes.

The study was conducted in a manner that yields quantitative information on the various discrete processes involved. This was accomplished by the simultaneous measurement of the renal plasma clearance of the drug and the rate of glomerular filtration under different experimental conditions.

Measurements were also made on the extent to which sulfamerazine is bound to the non-diffusable constituents of plasma, presumably plasma albumin, since it is only the unbound drug in the plasma water that is presented to the glomeruli for filtration. The ratio of the unbound drug clearance to the concurrent rate of glomerular filtration (excretion ratio) yields information on the extent to which the filtered material is reabsorbed or excreted by the renal tubules.

MATERIALS AND METHODS[2]

Experimental procedures. The human studies were carried out in young adult patients with normal renal function. Mannitol was used for the measurement of glomerular filtration rate (7). Simultaneous sulfonamide and mannitol clearances were determined at constant plasma concentrations of each during 3 consecutive periods of approximately 15 minutes. Urine was collected by means of an indwelling catheter with bladder washing. Blood samples were obtained at the mid-point of each urine collection period.

The dog experiments were performed on 3 well-trained female mongrels. The glomerular filtration rate was measured by the creatinine clearance (8). The sulfamerazine clearance and glomerular filtration rate were determined simultaneously for several collection periods. Various procedures designed to alter the excretion of the drug were then introduced and the observations continued.

Blood was obtained during each experiment for the estimation of total plasma protein and albumin content so that the concentration of unbound sulfonamide in the plasma water could be determined and its clearance calculated. The proportion of total plasma sulfonamide bound on the non-diffusible constituents of plasma was determined by dialysis across a cellophane membrane against an isotonic phosphate buffer at pH 7.4 and 37° C. Eighteen hours were allowed for the establishment of an equilibrium. All values for plasma binding were corrected to a standard plasma albumin content of 4.0 grams per 100 ml. This correction assumes that the plasma albumins of different individuals have no qualitative differences in their abilities to bind sulfonamides. Each experiment was performed in duplicate, at 4 different concentrations of plasma sulfonamide.

Chemical methods. The concentration of mannitol in the plasma and urine was determined by a modification (9) of (7); creatinine by a modified Folin procedure on tungstic acid filtrates (10); and sulfonamides by the method of (11). Twenty per cent p-toluene sulfonic acid was used as the precipitating and hydrolyzing agent in the determination of the N^4-acetyl sulfamerazine. Chloride in the urine was estimated by a modification of the Volhard method (12). Total plasma protein and albumin were determined by a micro-Kjeldahl method (13) after precipitation by the Howe technic (14).

RESULTS

Plasma binding. The values for plasma binding of the 3 sulfonamides studied (sulfamerazine, N^4-acetyl sulfamerazine, and sulfadiazine) are

[1] This work has been aided by a grant from the John and Mary R. Markle Foundation.

[2] The sulfamerazine and N^4-acetyl sulfamerazine were generously supplied by Sharpe and Dohme Company.

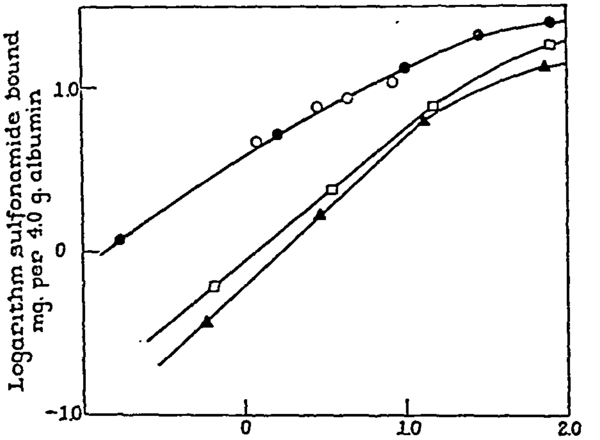

FIG. 1. RELATION BETWEEN BOUND AND FREE FRACTIONS OF SULFAMERAZINE, N[4]-ACETYL SULFAMERAZINE, AND SULFADIAZINE IN HUMAN PLASMA AND OF SULFAMERAZINE IN DOG PLASMA

All values are corrected to a standard plasma albumin content of 4.0 grams per 100 ml. The data are recorded logarithmically for the sake of convenient presentation. The actual whole plasma concentrations of the sulfonamides ranged from approximately 1 to 100 mgm. per cent. The sulfamerazine curve for human plasma represents the average of 3 experiments; the other curves are derived from single experiments.

● = Sulfamerazine, human plasma
○ = N[4]-acetyl sulfamerazine, human plasma
□ = Sulfadiazine, human plasma
▲ = Sulfamerazine, dog plasma

somewhat lower than previously reported (15). Calculations from the present data (Figure 1) indicate that 63 per cent of the sulfamerazine is bound at a whole plasma concentration of 10 mgm. per cent, whereas only 32 per cent of sulfadiazine is bound at the same concentration. The binding of the N[4]-acetyl sulfamerazine is essentially the same as the parent complex. It may also be noted that there is more extensive binding of sulfamerazine in human than in dog plasma.

Renal excretion of sulfonamides by man. The renal excretion of each of the 3 sulfonamides studied was examined in 4 young adults with glomerular filtration rates within normal limits (16). These data are summarized in Table I.

The renal plasma clearance of sulfamerazine in man is very low, the average for the 4 subjects examined being only 3.9 ml. per minute. This is considerably lower than in the dog (No. 1). The clearance of sulfadiazine is higher and more variable, ranging from 11.6 to 33.9 ml. per minute in the 4 subjects studied, while that of N[4]-acetyl sulfamerazine is still greater having a mean value of 52.4 ml. per minute.

The data on the excretion ratios (last column, Table I) indicate that an average of 65 per cent of filtered sulfadiazine and 85 per cent of sulfamerazine is reabsorbed by the renal tubules. The N[4]-acetyl sulfamerazine, however, would appear to be excreted in part by a process of renal tubular excretion, as is indicated by the high excretion ratio of 2.42.

Renal excretion of sulfamerazine by the dog. The very low excretion rate of sulfamerazine suggests that its reabsorption by the renal tubules is, at least in part, the result of the operation of an active transport system. It was because of this possibility that the study was extended to include observations on the dog which might be expected to yield information on the mechanisms involved. The variables examined include the rates of reabsorption of water and electrolyte by the renal tubules, and plasma drug level. In addition, an attempt was made to block the possible active transport system in the tubule cells with other substances that might compete with the sulfamerazine.

Experiments in which the tubular reabsorption of water was decreased by the intravenous administration of sodium sulfate and increased by the administration of pituitary extract are summarized in Table II. The sodium sulfate experiments are complicated by some increase in glomerular filtration rate (Expts. 1 and 2). However, diuresis in each case was accompanied by proportionately higher increases in sulfamerazine clearance, with consequent increases in its excretion ratio. In contrast, increases in sulfamerazine clearances were also obtained when water diuresis was interrupted by the administration of posterior pituitary extract (Expts. 3 and 4). It is impossible, therefore, to derive a simple relationship between renal tubular reabsorption of water or the rate of urine flow and the excretion ratio of sulfamerazine. Such a circumstance does not preclude the possibility that some sulfamerazine is reabsorbed by a passive process. It does minimize the probable importance of such a process in determining overall excretion since in

this case there should be some correlation with the rate of urine flow.

Diuresis effected by sodium sulfate and antidiuresis by posterior pituitary extract are typically associated with increased electrolyte excretion (Expt.4, Table II). A possible correlation between the reabsorption of electrolyte and the extent of the reabsorption of sulfamerazine was further examined in experiments wherein sodium chloride, potassium chloride, and ammonium chloride were orally administered. These experiments are summarized in Table III. Of these, only sodium chloride administration was accompanied by a dramatic increase in chloride excretion, although there was a general elevation in all, and electrolyte excretion was undoubtedly increased in each instance. There was an observable decrease in reabsorption of sulfamerazine in each experiment, which is also true after sodium bicarbonate (17). However, significant quantities of sulfamerazine were still reabsorbed at the higher chloride excretion rates.

Experiments designed to demonstrate the participation of an active transport system in the tubular reabsorption of sulfamerazine were unsuccessful. Studies on the filtration, excretion, and reabsorption of sulfamerazine over a wide range of plasma drug concentrations (2 to 43 mgm. per cent) gave no evidence of a maximum rate of reabsorption which is a common characteristic of active transport systems. Nor was it possible to block the reabsorption of sulfamerazine to a significant extent by the continuous intravenous administration of maximally tolerated amounts of amino acids (casein hydrolysate) and benzoic acid. These materials were chosen because of possible similarities in chemical structure to sulfamerazine.

DISCUSSION

The addition of a methyl group in the 4-position of the pyrimidine ring of sulfadiazine, to form sulfamerazine, results in a compound that is extensively reabsorbed by the renal tubules and, therefore, has a very low rate of renal excretion. It is somewhat surprising to find that acetylation of sulfamerazine at the N^4-position results in a compound that is excreted rather than reabsorbed by the renal tubular cells.

The low excretion rate of sulfamerazine is the result of two factors. Sulfamerazine is bound in human plasma to such an extent that, at a whole

TABLE I

The renal excretion of sulfamerazine (SM), N^4-acetyl sulfamerazine (ASM), and sulfadiazine (SD)

The clearance values are corrected to a standard surface area of 1.73 sq. m. The clearance and ratio values are the average of 3 consecutive 15-minute periods. The clearance ratio is calculated from the whole drug clearance and the glomerular filtration rate; the excretion ratio, from the unbound drug clearance and the glomerular filtration rate.

Patient	Drug	Range of urine flow	Range of whole plasma drug level	Average glomerular filtration rate	Average whole plasma drug clearance	Average clearance ratio	Average excretion ratio
		ml. per minute	*mgm. per cent*	*ml. per minute*	*ml. per minute*		
Se	SM	3.4 to 3.6	4.0 to 4.9	109	3.61	0.033	0.154
Ch	SM	1.8 to 2.4	10.8 to 11.8	104	4.18	0.040	0.155
Sc	SM	2.5 to 3.0	4.8 to 5.0	110	4.03	0.037	0.164
Ga	SM	2.3 to 2.9	4.5 to 5.2	117	3.63	0.031	0.129
				Average:	3.86	0.037	0.150
Ju	ASM	3.8 to 4.4	2.2 to 2.7	117	53.2	0.455	2.23
Yo	ASM	2.0 to 2.2	1.7 to 1.9	103	59.6	0.580	2.77
Sc	ASM	3.4 to 3.7	2.7 to 2.8	107	33.6	0.314	1.59
De	ASM	2.2 to 2.7	2.2 to 2.2	103	63.0	0.613	3.11
				Average:	53.4	0.490	2.42
Bi	SD	1.8 to 2.4	3.3 to 3.4	148	19.1	0.132	0.265
Mc	SD	8.0 to 8.8	4.5 to 4.5	141	33.9	0.242	0.442
Ma	SD	2.8 to 3.1	3.9 to 4.2	106	29.4	0.276	0.494
Mr	SD	2.0 to 2.1	4.8 to 5.5	108	11.6	0.107	0.189
				Average:	23.5	0.189	0.348

TABLE II

Effect of variation in urine flow on the excretion rate of sulfamerazine (SM) in the dog

The values for the concentration of sulfamerazine free in the plasma water were obtained by correcting the whole plasma concentrations for the amount of drug bound on plasma proteins.

Expt. No.	Dog No.	Urine flow	Glomerular filtration	SM plasma water level	SM plasma water clearance	Excretion ratio	Chloride excretion
		ml. per minute	*ml. per minute*	*mgm. per cent*	*ml. per minute*		*m.eq.×10⁻³ per minute*
1	1	3.89	66.0	4.06	11.4	0.17	
		1.90	61.1	4.00	10.4	0.17	
400 ml. 6 per cent sodium sulfate, intravenously							
		13.55	83.4	2.57	32.0	0.38	
		9.00	82.4	2.75	28.2	0.34	
2	2	0.63	55.2	6.70	2.98	0.05	
		0.28	55.4	6.78	4.15	0.08	
500 ml. 10 per cent sodium sulfate, intravenously							
		9.24	69.0	5.33	16.0	0.23	
		12.34	66.7	4.94	18.0	0.27	
		11.58	65.0	5.14	18.8	0.29	
3	1	6.00	84.7	4.14	11.9	0.14	
		6.70	79.2	4.11	13.4	0.17	
10 units posterior pituitary extract, subcutaneously							
		0.28	73.1	3.88	12.5	0.17	
		0.53	78.3	3.65	19.5	0.25	
		0.76	76.9	3.64	19.9	0.25	
4	2	5.90	59.0	2.69	15.8	0.27	16.6
		4.95	55.2	2.78	14.9	0.27	10.4
10 units posterior pituitary extract, subcutaneously							
		1.27	61.4	2.95	24.1	0.39	95
		1.67	60.2	2.86	25.7	0.43	150
		1.42	62.2	2.85	26.6	0.43	119
		1.22	64.4	2.79	29.7	0.46	114

plasma level of 10 mgm. per cent, only 40 per cent is unbound in the plasma water and is available for filtration at the glomeruli and thus for excretion. The reabsorption of sulfamerazine by the renal tubules is equally important, being on the order of 85 per cent of the amount filtered.

The low overall excretion rate of sulfamerazine has two important consequences. First, less frequent and smaller doses of sulfamerazine than of sulfadiazine are required to maintain any given plasma level (2 to 6) since the clearance ratio of sulfamerazine is only one fifth that of sulfadiazine. Also, it would be supposed that the renal hazard incidental to the precipitation of insoluble sulfonamide compounds in the genito-urinary tract would be less as compared to other sulfonamides with higher excretion rates. For example, the urine concentration of sulfamerazine when its plasma concentration is 10 mgm. per cent and the urine flow is 1 ml. per minute may be calculated to be 38.6 mgm. per cent whereas the urine concentration of sulfadiazine in a comparable situation would be 235 mgm. per cent.[3]

[3] Drug clearance (ml. per minute) = UV/P, where U = concentration of drug in urine in mgm. per cent, V = urine flow in ml. per minute, and P = concentration of drug in plasma in mgm. per cent.

TABLE III

Effect of electrolytes on the excretion of sulfamerazine (SM) in the dog

The values for the concentration of sulfamerazine free in the plasma water were obtained by correcting the whole plasma concentrations for the amount of drug bound on plasma proteins.

Expt. No.	Dog No.	Urine flow	Glomerular filtration	SM plasma water level	SM plasma water clearance	Excretion ratio	Chloride excretion
		ml. per minute	*ml. per minute*	*mgm. per cent*	*ml. per minute*		*m.eq.* $\times 10^{-3}$ *per minute*
5	1	7.8	74.5	1.94	16.3	0.22	60
		7.2	65.2	2.20	16.2	0.25	52
		6.3	61.5	2.35	15.2	0.25	42
200 ml. 1.8 per cent sodium chloride, *per os*							
		2.3	68.7	2.58	27.7	0.40	353
		2.7	71.2	3.05	27.3	0.38	419
		2.4	78.0	2.58	26.5	0.34	420
6	3	6.5	102	2.90	16.1	0.16	41
		5.9	91.8	3.71	15.0	0.16	21
400 ml. 1 per cent potassium chloride, *per os*							
		7.8	107	3.55	30.9	0.29	36
		7.3	102	3.42	27.4	0.27	59
		7.6	102	3.30	35.5	0.35	176
7	1	4.5	56.3	2.19	14.9	0.26	18
		4.8	59.5	2.50	13.0	0.22	21
400 ml. per cent ammonium chloride, *per os*							
		3.0	55.0	2.76	20.3	0.37	23
		3.2	55.4	2.66	22.4	0.40	31
		3.4	56.3	2.64	21.4	0.38	42

The somewhat greater solubility of sulfamerazine as compared to sulfadiazine (18) should serve to further decrease the renal hazard. However, no striking differences have been noted in the incidence of renal complications during the therapeutic use of these two drugs in man (3 to 6). A recent report (19 indicates that renal calculi may occur more frequently with sulfamerazine than with sulfadiazine therapy. However, the difference in the incidence of renal complications with the two drugs was not significant at equivalent plasma concentrations. It is possible that more extended clinical observation, when all factors affecting renal excretion are controlled, will reveal a real difference in the renal hazard due to sulfamerazine as compared to sulfadiazine. The extent to which the high excretion rate of the N^4-acetyl derivative of sulfamerazine contributes to the renal hazard is not known but may be significant.

It would be of considerable theoretical interest to define the mechanisms concerned in the renal excretion of sulfonamides such as sulfamerazine. Data are presented which indicate that the reabsorption and excretion of sulfamerazine are not specifically related to the rate of urine flow, but may be in some way related to the renal tubular mechanisms concerned with the reabsorption and excretion of electrolytes. Procedures which increase electrolyte excretion also appear to decrease the tubular reabsorption of sulfamerazine and thus result in an increased rate of drug elimination. The increase may be considerable and occurs in a variety of circumstances. Thus, the administration of posterior pituitary extract, sodium, potassium, and ammonium chloride, and sodium bicarbonate (17) are effective in the dog. It is likely that a similar situation obtains in man, particularly since it has been observed that man excretes sulfadiazine at an increased rate when

given sodium chloride or bicarbonate (20). It has been reported that sulfadiazine excretion is related to the pH of urine (21) but it seems likely that this may be related to the disturbance in electrolyte excretion which accompanies a change in urine pH as well as to the change in pH itself.

The effect of a disturbance in electrolyte excretion on the elimination of sulfamerazine has obvious therapeutic implications. It is common practice to administer sodium bicarbonate to patients receiving sulfonamides to increase their solubility in urine. This procedure will also increase the excretion rate of the drug. Decreased chloride excretion is commonly observed in lobar pneumonia and in severe burns and will be accompanied by diminished rates of sulfonamide excretion. Careful observation of drug plasma levels is indicated in these conditions.

Initial attempts to demonstrate the active renal tubular reabsorption of sulfamerazine in the dogs' kidneys have been unsuccessful. However, there is indirect evidence which suggests that this is the case. It is difficult to understand how passive diffusion across the tubule cells can account for the reabsorption of as much sulfamerazine as indicated by the average excretion ratio of 0.15 (Table I). This view is emphasized when it is considered that such a relatively simple and soluble compound as urea (which is passively reabsorbed) has an excretion ratio of approximately 0.6.

SUMMARY AND CONCLUSIONS

1. The addition of a methyl group to the pyrimidine ring of sulfadiazine to form sulfamerazine results in a compound that has a very low overall renal excretion rate. This is the result of extensive reabsorption by the renal tubules and binding on plasma proteins.

2. The N^4-acetyl derivative of sulfamerazine (which is presumably its conjugated form) is secreted rather than reabsorbed by the renal tubules.

3. The low overall excretion rate of sulfamerazine has two distinct therapeutic advantages. First, relatively infrequent and small doses are required to maintain any given plasma concentration. Second, the urine concentration of the drug at any given plasma level is less than that of other sulfonamides in current use. Such a circumstance should minimize the renal hazard of sulfonamide therapy, but additional factors such as solubility, urine flow, and pH may be expected to operate in this respect.

4. An increase in the excretion rate of sulfamerazine accompanies augmented electrolyte elimination. This occurs when the electrolyte excretion is increased by a variety of means. The renal excretion of sulfamerazine does not appear to be simply related to the rate of urine formation.

5. Indirect evidence suggests that sulfamerazine is reabsorbed by an active transport system in the renal tubules but this has not yet been directly demonstrated.

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PROCEEDINGS OF THE THIRTY-SIXTH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION HELD IN ATLANTIC CITY, N. J., MAY 8, 1944

READ BEFORE THE SCIENTIFIC SESSION

PRESIDENTIAL ADDRESS

SOME OF THE "DO'S" AND "DO-NOT'S" IN CLINICAL INVESTIGATION

By FULLER ALBRIGHT

When your President and Council decided to hold this meeting, they had to ask themselves the following question put to them by the Office of Defense Transportation in Washington: Is this meeting likely to contribute to the over-all war effort? I, for one, and I think I speak for the majority of the Councillors, had no hesitation in answering the question in the affirmative. All knowledge is interrelated; in times of stress the most scientific nation has a big advantage. Surely a society like ours, where year in and year out some of the most important advances in medicine have first come to the light of day, should carry on through the present unpleasantness. Moreover, with so many of our colleagues scattered over the 7 seas, a big responsibility accrues to us who are left to keep the home fires burning in respect to clinical investigation.

It is perhaps a presumptive symptom of an oncoming intellectual menopause,—indeed, one might say it is evidence of a Young Turk becoming an Old Turk,—when one endeavours to lay down certain precepts for success in a field. It is probably fortunate that no one follows such precepts anyway, that each prefers to learn his own way, though this be the hard way. Be all this as it may, as I look around at those of our colleagues who have attained success in the field of clinical investigation and analyze what methods they have used, I see certain recommendations or "Do's" which may be worth jotting down; furthermore, as I look further, especially into my own past, I see certain "Do-not's" which may be equally worthwhile jotting down as practices to be avoided. I won't attempt to define "success." I do not necessarily mean academic recognition; I do not necessarily mean self-satisfaction; I just mean success.

First let me insert a short digression on what is meant by "clinical investigation." I recently had the pleasure of sitting in on a discussion in which our editor-in-chief, Dr. James L. Gamble, deplored the term "sub-clinical" used in the sense of "pre-symptomatic"; Dr. Chester S. Keefer, who was present, enlarged on this theme and pointed out that "clinical" is derived from the Greek word "klinikos" meaning bed, and that "sub-clinical" (a Latin-Greek hybrid) literally means "under-the-bed." Since animals do not sleep in beds it is quite clear that "clinical investigation" has primarily to do with the investigation of sick people, and is concerned only secondarily, if at all, with sick laboratory animals. But more of that in a minute.

I think of a clinical investigator as one trying to ride two horses,—attempting to be an investigator and a clinician at one and the same time. Whereas such an equestrian manoeuver is usually considered a bad policy, in this case, probably because of two considerations in particular, experience has shown that it is a very fruitful pastime. In the first place, the ultimate goal of most investigation is to find something of benefit to the human race; where, other than by the bedside of sick patients, could one find so many suggestions of things to be investigated? Secondly, in many instances, nature has arranged an experiment in a sick individual and partly completed it; all that is needed are the eyes of the clinician to make certain observations, and the background of the investigator to plan other observations and interpret them.

This rider of two horses, however, must remember that there are two horses; he must avoid the danger on one side that he, as a clinician, be swamped with patients and the equal danger on the other side that he, as an investigator, be segregated entirely from the bedside. In his laboratory, necessarily easily accessible to the wards, his clinical half will be constantly interrupted by such messages as that Mr. Humpty-Dumpty has had a big fall. As a result, his investigative half will find that he cannot compete with the straight non-clinical investigators as regards animal or smoked-drum experiments. To answer a vital question, where such technics are necessary, it is often preferable that he persuade one of his non-clinical colleagues to carry out the observations. You can all look around you and see many examples where a good clinician has gone to the laboratory to get the answer to a question and has gotten it,—more power to him. All I am trying to say is that, if you have acquired the difficult technic of being a fair clinician, you had better use this technic in your clinical investigation.

Let me add one additional point: an intelligent patient, private or otherwise, to whom you have taken the trouble to explain the nature of the investigation, makes the best laboratory animal.

I would not feel quite right addressing this Society without presenting one of what Dr. James H. Means has termed, because of the arrows, my St. Sebastian diagrams. Accordingly, I have arranged one such (Figure 1), depicting the "Do's" and the "Do-not's" which one must pass by in climbing the road which leads to the Castle of Success in Clinical Investigation. You will note that the road is formed by the amalgamation of two paths,

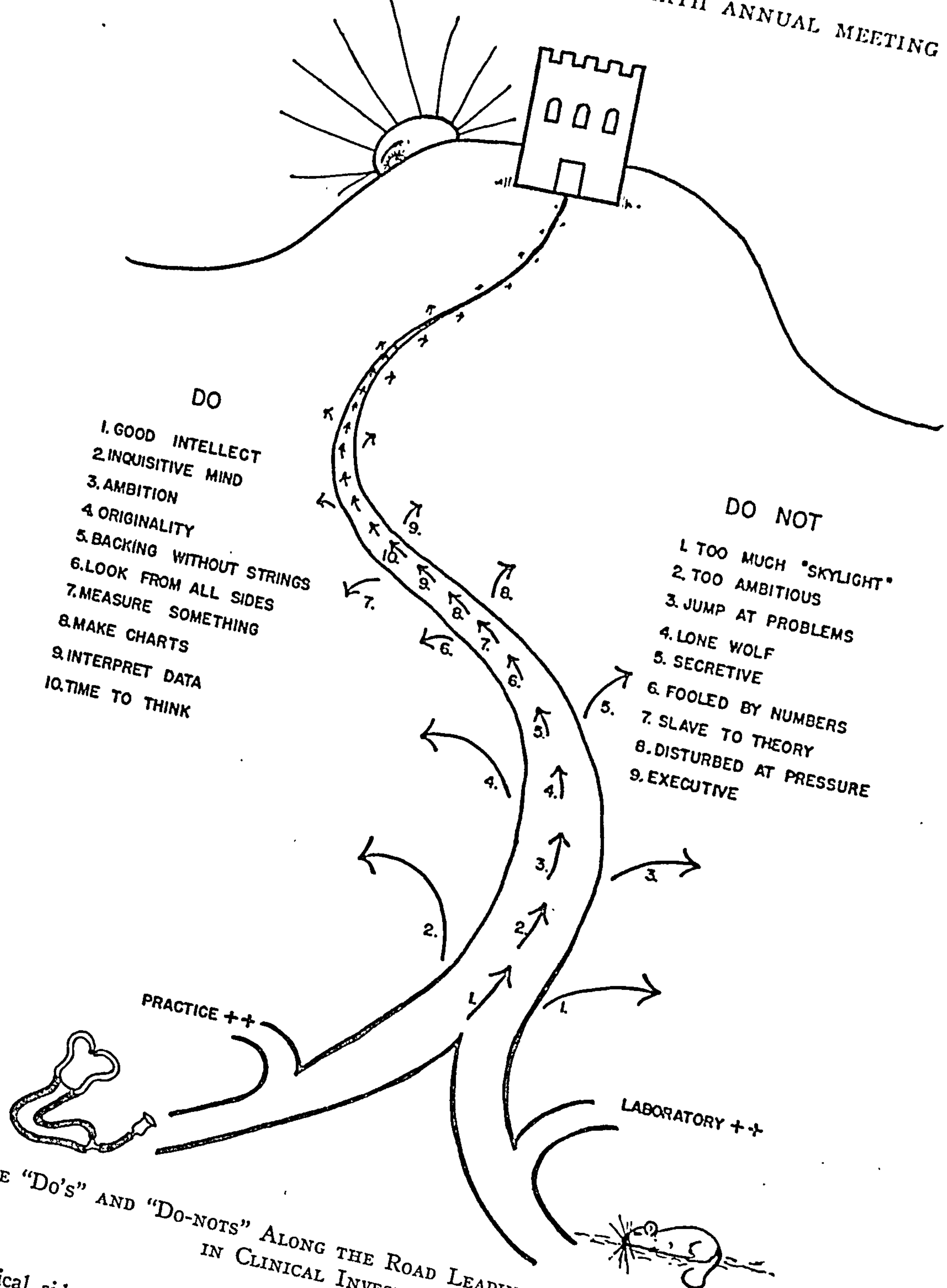

FIG. 1. THE "DO'S" AND "DO-NOTS" ALONG THE ROAD LEADING TO THE CASTLE OF SUCCESS IN CLINICAL INVESTIGATION.

one representing the clinical side and the other the laboratory side.

"*Do*" *No. 1.* Do be born with a good intellect, not necessarily the best of intellects. Oliver Wendell Holmes divided minds into "one-story intellects, two-story intellects, and three-story intellects with skylights." "All fact-collectors," he noted, "who have no aims beyond their facts, are one-story men. Two-story men compare, reason, generalize, using the labors of fact-collectors as well as their own. Three-story men idealize, imagine, predict; their best illumination comes from above through the skylight." One sees the same types among clinical investigators; all three have their places. We all know the data collectors, who fill the literature with amorphous data but do not tell us what the data mean. We can look around us, too, and see a few three-story intellects with skylights. One such was the late Dr. L. J. Henderson. It is often unnecessary for the highest intellects to do any collecting of data; they can often take somebody else's published data and arrive at some important new truth. This requires no laboratory animals, no complicated chemical determinations. In this connection,

Dr. Read Ellsworth once cited an excellent example. Throughout nearly an entire lifetime, Tycho Brahe sat in his laboratory assiduously observing and recording, night after night, the precise position of the planets and stars. Kepler, a calculator par excellence, seized upon this amorphous mass of figures and out of the confusion there emerged three great laws of the movement of the planets. This fortuitous combination of Tycho Brahe's patient accuracy with Kepler's ability to reason and calculate, the one-story intellect with the three—, turned out to be extremely significant for Science.

"Do-not" No. 1. But, do not have your skylights too widely open; install some venetian blinds. There is a real danger in having more mind than matter, as Dr. E. Cowles Andrus aptly put it,—of having so many ideas that one flits from problem to problem without ever completing anything. After perhaps ten years of flitting, our intellectual genius wakes up to find that a one-story intellect friend, who has had but one thought, has proved it beyond all manner of doubt while he himself has had innumerable brilliant theories all of which still remain theories.

"Do" No. 2. Do develop an inquisitive mind. Some people can look at a problem and never see that there is a problem; in the words of the late Professor Jacob Erdheim, with whom I spent a very profitable year, "Die Augen sind gut, aber sie schauen nicht an." Professor Erdheim was constantly asking himself, and surprisingly often answering the question: "Wass is die Ursache?" If you don't ask the questions, you won't find the answers. For example, most doctors would look at a patient with Paget's disease with bowing in one leg and not the other and would fail to ask themselves why the bowed leg is long enough to reach the ground,—in fact is as long if not longer than the other leg. If one asks the question, as did Professor Schmorl of Dresden, and looks for the answer, the explanation is simple and quite interesting (1).

"Do" No. 3. Do be ambitious. Ambition breeds energy. Clinical investigation requires sweat, if not blood and tears!

"Do-not" No. 2. Do not be too ambitious. Too-much-ambition breeds jealousy; jealousy breeds unhappiness. At any one time, credit seldom goes where credit is due. When the partition of credit leaves our over-ambitious colleague on the short end, he boils. Let our unhappy colleague whose work has produced important negative evidence keep his sense of humor; his more discerning colleagues recognize that, in the labyrinth of Science, to point out that one door after another is not the right one is of great help to those who follow. With many intricate problems, it is necessary first to demonstrate that two and two do not make five or six or seven, before the fellow who gets all the credit comes along and discovers that they make four. In short, let our colleague remember that, in the long run, credit does go where credit is due.

"Do" No. 4. Do have that something which, for want of a better term, I will designate "originality." One can possess the best of intellects and still fall down in investigation because one is completely lacking in this. I do not think that the prospective investigator, or his adviser, can tell without his actually trying whether this important but intangible quality is present. I am not sure that one cannot acquire it. I rather think that a well-developed ability to correlate ideas may almost take the place of what I am talking about.

If one makes it a practice every time one hears of a new and interesting fact to ask himself if this fact has any bearing at all on his own particular pet problem, it is surprising to find how often it may have. For example, one hears that phosphatase is found in the kidneys. The parathyroidologist asks himself: "Does this have anything to do with parathyroid function?" "Well," he muses, "the parathyroids have something to do with excretion of phosphates in the urine. Phosphatase has something to do with getting phosphates across membranes. Ergo, does the parathyroid hormone influence the amount of phosphatase in the kidneys?"[1] The final question may have the appearance of an original thought when as a matter of fact it is the logical outcome of a process of correlation,—of putting a few known facts in juxtaposition as it were.

"Do" No. 5. Do obtain a backing, financial and otherwise (Don't ask me where!), which leaves you absolutely free to pursue whatever project seems most promising. The man and not the project should be endowed. His natural interest may take him from calcium to parathyroids, to bone disease, to kidney stones, to urinary infections; from calcium to bone disease, to gonadal hormones, to ovarian dysfunction, to amenorrhea, to hypothalamus, to psychosomatic medicine, etc., etc. Too often promising work in one field is interrupted because somebody with a bank account wants to know the answer to some specific problem such as the cure for cancer, the cause of otosclerosis, or what have you. New knowledge, be it where it may, is of importance, and the cure for cancer may come from somebody who is working in a field apparently entirely unrelated to cancer. I am told that the Russians are encouraging scientific research of any kind. Certainly from the 1940–41 programs of the Biochemical Society of London one would never suspect that England was in a life-or-death struggle.

"Do" No. 6. Do look at your problem from all points of view; don't get too close to it at first, but cover the entire field with a low-power lens; then when some point of interest presents itself turn down on that with a high-power lens.

Most problems in clinical medicine are best approached from several directions. Take the subject of hyperparathyroidism. The clinical side tells us that some cases have such and such symptoms in relation to their skele-

[1] This series of questions was stimulated by the interesting investigations of Dr. Charles D. Kochakian of Rochester, N. Y. He went through the same process of reasoning and is now in the process of obtaining the answer to the final question.

tons, that others have no skeletal symptoms, that kidney stones and polyuria are common, whether or not skeletal symptoms are present, etc. The morbid anatomy of the bones shows us that there is a very rapid turn-over of bone tissue in those cases that have bone disease and that there is absolutely nothing abnormal going on in the bones in those cases that do not have bone disease. The chemical side tells us that the level of serum calcium is high and the level of serum phosphorus is low whether there is bone disease or not, but that the serum phosphatase level is high only in those cases with bone disease, etc. The metabolic studies tell us that the urinary calcium and phosphorus excretions are high in all cases whether they have bone disease or not, that a patient need not be in negative calcium balance if the calcium intake is sufficiently high, etc. But the combined approach, which takes in clinical aspects, morbid anatomy, chemical findings, and metabolic observations, leaves us with some idea what the disease is all about.

"Do-not" No. 3. Do not jump at the first problem which presents itself. When I first started investigation, I thought that it would be virtually impossible to find a problem which had escaped the attention of eager investigators since the beginning of time and which gave any hope of being soluble. As a matter of fact, there are good problems everywhere. Remember, it is much easier to start an experiment than it is to finish one. This is one lesson that I have never been able to learn; I hate to think of the grief I would have been spared had I learned to count ten before launching into a new problem.

There are certain reservations, however, which should be made to the above. In clinical investigation it is often necessary to start a new problem on the spur of the moment in order to employ some unusual opportunity produced by disease. A reverse reservation is that certain problems should never have been started. And here it should be sadly but firmly recognized that it is a mistake to go on and on in the hope of salvaging something rather than to scrap the whole wretched business.

"Do-not" No. 4. Do not be a lone-wolf investigator, —one who never discusses his results or methods of approach with anybody else, who never invites criticism. Most problems are sufficiently complex that they require for their solution the combined efforts of a group. This group need not necessarily be set up as a group under one chief; one investigator working by himself in his own laboratory is not a lone-wolf investigator if he frequently seeks the advice and help of his colleagues in other laboratories—if he makes it a practice to attend the "May meetings" in Atlantic City, and puts in some good work comparing notes on the boardwalk.

"Do-not" No. 5. Do not be secretive. Talk about your work without fear that somebody will publish before you. The danger one runs of having intellectual property pirated is far offset by the suggestions one receives from colleagues. I recall waiting for the report of certain experiments, the details of which were kept secret; when the report finally came out it was full of loopholes which might have been eradicated had the authors discussed their findings with others. I am quite sure that nine times out of ten, when an investigator believes that his work has been pirated, this is not the case. Knowledge accrues usually in a logical manner. The next step comes when the stage has been set. It is not surprising when two or three laboratories arrive at the same answer at the same time. Material which is rushed into print for the sake of priority is usually from the hands of one-story intellects, trying to beat other one-story intellects in reporting some perfectly obvious experiment.

Furthermore, half the fun of investigation is talking about it. If one talks only about the experiments one has completed and proved, one will have relatively little conversation; if one talks about all the theories one has not yet proved, the sky's the limit.

"Do" No. 7. Do measure something. I need not remind you gentlemen that science is based on measurements. Indeed this is so well-understood today that at times one wonders whether the pendulum has not swung too far from the state of affairs in the Middle Ages. Then it was all metaphysics and no measurements; now it is all measurements and no metaphysics. Perhaps a dash of the latter would be a useful condiment.

The real problem is what things to measure, how to measure them, and especially what control measurements to make. I have no special thoughts on this subject other than one minor point. In general, one should make the measurements to answer the problem; not look for a problem which will be an excuse for carrying out certain complex measurements. This assertion must of course be qualified. An interesting new investigative tool is discovered (e.g., isotopes); it is certainly intelligent to do a bit of prospecting with this new tool in order to determine where in the investigative field it will produce additional information. Some investigators are born gadgeteers; they love complicated set-ups. Some of the best advances, however, have been made with very simple measurements. The determination of the weight of hair produced in an axilla, expressed in grams per axilla per week, may give you the same information, mirabile dictu, as an assay of the urinary excretion of that steroid which has a ketone group on the seventeenth carbon atom.

"Do-not" No. 6. Do not be fooled by figures. Granted that figures do not lie and that liars figure, the fact remains that figures can give you a false sense of security. One can prove statistically that one set of figures is significantly different from another set; the question remains as to why they are different. No matter how careful you are, in almost any experiment another variable than the one you are studying creeps in and may be the cause of the changes observed. In studies on calcium metabolism, for example, the unaccounted for variable may be the amount of ultraviolet light in the atmosphere or the amount of calcium swallowed with the tooth powder.

"Do" No. 8. Do where possible arrange your data in chart form. This has long been a recognized practice with certain variables,—especially temperature, pulse, and respiration. The importance of charting was first brought home to me at Johns Hopkins where Dr. Warfield T.

Longcope has his students chart any variable that can be charted. I remember particularly a case of undulant fever with intermittent hydrarthrosis where Dr. Benjamin M. Baker, Jr. charted the circumference of each knee and found that the right knee was swollen when the left knee was down and *vice versa* in perfectly regular 7-day cycles (2). These are very interesting observations which probably would never have been made had the data been entered in the record as so many separate notations. Incidentally, these observations of Dr. Baker have been in the literature now for 15 years. It is about time some inquisitive mind put forth a theory as to why one knee was swollen when the other was down and why the cycles were exactly 7 days. I can hear Erdheim's spirit mumble: "Wass is die Ursache?"

In the preparation of data for publication, I also believe a chart is extremely helpful to the reader; it should not, however, replace tables of data, which are much more convenient for the three-story intellects who will usually wish to make some recalculations.

"Do" No. 9. Do develop a theory or, at the very least, do try to correlate your work with the sum total of human knowledge. Don't burden this weary world with data without giving a hint as to the reason why they were collected and what they mean. As some of you know, I am one of those who believe that any theory is better than none at all. For example, I am not happy with the statement that, on administration of parathyroid hormone, the serum calcium rises and the serum phosphorus falls. I want to know whether the serum calcium rises because the serum phosphorus falls or whether the serum phospohrus falls because the serum calcium rises, and what is more, I do not want the authors to straddle the issue.

The purpose of a theory is twofold: (*a*) to give you something upon which you can hang the facts, and (*b*) to give yourself and your colleagues something to tear down and replace with a better version. By "theory," you will note, I really mean "working hypothesis."

"Do-not" No. 7. Do not be a slave to your theory. When new facts present themselves which require a change in the theory, change the theory. Do not feel hurt if somebody else changes it for you. Do feel hurt if your facts are wrong; not if your theories are wrong.

"Do-not" No. 8. Do not be too disturbed at pressure exerted upon you to produce tangible results, *i. e.*, papers or reports. Often one hears lamentations over the "constant pressure to produce something." Such pressure has its obvious faults, but may be a blessing in disguise. Often, there is a certain amount of inertia about actually working up one's data and yet, not infrequently one's best thoughts do not come until one attempts to put down one's findings in black and white. Alas, it is too often only then that one really finds the shortcomings of one's data! Furthermore, if one waits a period of years with the expectation of getting out a magnus opus when the work is completed, one should remember that worthwhile work is seldom completed. There is always just one more answer which the inquiring mind would like to have. Finally, the data become so voluminous that the mind shrinks from assembling them.

"Do-not" No. 9. See to it that you do not wake up some fine morning in an executive job. Do not show too much administrative ability. The first time you are asked to serve on a committee, be anything but efficient. Never make the mistake of proposing some new reform; you are apt to be chosen as a committee of one to put said reform through. The desk of the good executive should be clear; that of an investigator should be littered. Questions will constantly come up which cannot be immediately settled and filed away. They must be pondered over. Whatever else you do, do not become a Professor of Medicine or the head of a department. Let me make it clear that I do not deprecate the good executive. I realize that it may be commendable for a man to sacrifice his own investigative career to direct the investigations of others. He may climb to bigger and better castles but his chances of arriving at the castle under discussion lessen with his executive duties. I appreciate too that some men manage to continue their investigative work in spite of the fact that they become executives. I am cognizant that the top academic jobs are mostly executive and must be filled by the top academic men. I am aware of the fallacy of the often-made suggestion that the administrative jobs be filled by men with executive and not necessarily academic ability. I have little constructive criticism to offer at this point. An obvious suggestion is to find some Lady Bountiful to endow some purely research associate professorships in clinical departments. But I am not sure that this would work; there is such a thing as being too sheltered from the world of responsibility. Certainly such professorships should not be relieved of the responsibility and stimulation of teaching and care of patients.

There is another more subtle way to find oneself eased into an executive job. You start, shall we say, as a young investigator apprenticed to a seasoned veteran; perhaps a small corner of the laboratory is allotted to you where you can carry out your measurements. The first step forward comes perhaps when you get sufficient money from some fund to hire a technician. Then your work may attract the attention of some bright young investigator who attaches himself to you; then come more funds, more technicians, and more assistants. All the financial grants require yearly reports; data are collected which have to be gone over; papers have to be written; speeches have to be made; teaching has to be taught; pretty soon one is less and less in the laboratory and more and more at his desk. You are caught—the fun of directly carrying on investigation is not for you;—you are an executive.

"Do" No. 10. Do try to reserve some time during the day when you can do some unadulterated thinking. If you salvage a few minutes, you will be doing better than most. Some people, the peripatetic school, do their thinking while walking around; they bump into you in the corridors and get the reputation of being absent-minded. Some think while they are driving their auto-

mobiles; this has its disadvantages to the public at large. Some get a few minutes while walking to and from their work; this is excellent. But most seize a few relaxed moments while taking the morning hot shower; this may lead to a hot water shortage if some particularly difficult concept has to be thought through. Let thinking come where it will, the important thing is that time be found to put the new facts which have come before one's sensorium in juxtaposition to the old problems.

LAST LAP

Well, you do the "Do's" and you do not do the "Do-Not's"; you arrive at the door of the Castle of Success. You still need the key to open the door. The key stands for the personal equation. "But personality does not count in pure science," you say. That may be true, but Clinical Investigation is not a pure science.

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The Mechanism of Pyridoxine-Deficiency Anemia. By George Cartwright (by invitation) and Maxwell M. Wintrobe, Salt Lake City, Utah.

Pyridoxine deficiency has been studied in swine with special reference to the anemia and its relationship to protein metabolism. The anemia is microcytic and hypochromic in type and is accompanied by an elevated serum iron, hemosiderosis of the tissues, and bone marrow hyperplasia. Nevertheless studies on serum bilirubin, reticulocytes, icteric index, and urobilinogen excretion showed that there is no increase of blood destruction. The anemia appears to be due to faulty synthesis of hemoglobin. The ferremia and hemosiderosis are prevented by restricting the dietary intake of iron. This indicates that the hemosiderosis is caused by the continued retention of iron at a time when its utilization for hemoglobin formation is at a minimum.

The place in hemoglobin formation at which pyridoxine exerts its action was sought by feeding a variety of substances, including chlorophyll, and hemin, to pyridoxine-deficient animals. The metabolism of tryptophane is disturbed in pyridoxine deficiency and three products of tryptophane metabolism are found in increased quantities in the urine of deficient animals. The significance of the finding of certain urinary pigments in cases of nutritional deficiency is discussed in relation to these observations and the factors concerned in hemoglobin formation are considered.

Panmyeloid Arrest in Rats Produced by a Purified Diet Containing Sulfaguanidine and Corrected by Liver or Yeast Extracts.[1] By Frank H. Bethell and (by invitation) Marion E. Swendseid, and Ray H. Rosenman, Ann Arbor, Mich.

Young mature rats maintained on a vitamin-free diet containing 2 per cent sulfaguanidine and supplemented by crystalline vitamins develop progressive leukopenia, granulocytopenia, thrombocytopenia, and normocytic anemia. It has been shown that these changes are the apparent result of a conditioned nutritional deficiency induced by the bacteriostatic effect in the intestine of the sulfanilamide derivative. The lowered values may be prevented or corrected by extracts of liver or yeast containing "folic" acid (vitamin B_c).

At the maximum observed developmental arrest, the marrow total nucleated cell count is but slightly less than that of normal rats. However, the majority of the marrow cells in the affected animals are undifferentiated primitive forms, with relative increases in myeloblasts and early erythroblasts. Myelocytes, late erythroblasts, and megakaryocytes practically disappear.

By means of marrow differential counts, performed on successive days after the administration of such liver or yeast extracts, it has been possible to determine the change in relative numbers of cells at each stage of development of the granulocyte and erythrocyte series. A close parallelism in the maturation of the members of the two cellular series is demonstrated. These observations provide evidence in support of the theory of a common stem cell origin of all blood cells developing in the marrow of the rat.

Studies on Increased Coagulability of the Blood. By Theo. R. Waugh and D. W. Ruddick (introduced by Donald McEachern), Montreal, Canada.

The need for a test to demonstrate the presence of an increased coagulability of the blood has been accentuated by the recent interest in thrombosis and the use of dicoumarol.

Such a test using heparin as an anticoagulant is here presented. By thus slowing the process, finer analysis of any abnormalities, particularly acceleration, is made possible.

The result is expressed as a graph and experimental evidence along with the theoretical consideration of the mode of action of heparin indicates that this method may be a measure of the relative thromboplastic content of the blood.

Studies employing this test demonstrate a small range of variability in normal individuals and an increased coagulability, (*1*) during uncomplicated bed-rest, (*2*) following operative procedures, and (*3*) in the presence of acute infections.

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[1] The liver extract was supplied by the Wilson Laboratories. The yeast extract was supplied by Parke, Davis and Company and contained 85 gammas of vitamin B_c per ml.

Response of Cardiovascular System of Normal Subjects to Acute Blood Loss.[1] By EUGENE A. STEAD, JR., and (by invitation) JAMES V. WARREN, EMMETT S. BRANNON, and ARTHUR J. MERRILL, Atlanta, Ga.

The removal of 300 to 900 ml. of blood was performed 10 times on 8 subjects in the basal state. Quantitative studies of the circulation were made before and after bleeding. A catheter was introduced into the right auricle, using the technic of Cournand and Ranges. The auricular pressure and samples of mixed venous blood for analysis of oxygen content were obtained by this means. A special inlying needle was placed in the femoral artery and the arterial pressure was recorded optically by the method of Hamilton and samples of blood obtained for analysis of oxygen content. The oxygen consumption was measured by the analysis of the expired air. From these data, the cardiac output was calculated, using the Fick principle.

In 5 experiments, the subjects appeared to be relaxed and the observations before bleeding were in the range of those of our normal basal subjects. The cardiac index (cardiac output in liters per minute per square meter of body surface) varied from 2.3 to 3.4. In 5 experiments, the subjects were anxious and the control observations showed evidence of a hyperactive circulation, the cardiac index ranging from 3.9 to 6.0. The high cardiac index was accompanied by only a slight rise in oxygen consumption. There was no observable difference in the response of these two groups to bleeding. The most striking change was a fall in auricular pressure, which ranged from 20 to 50 mm. of H_2O. Oxygen consumption, cardiac output, arteriovenous oxygen difference, mean arterial pressure, and calculated peripheral resistance showed no consistent changes in the 7 experiments in which circulatory collapse did not develop. In 4 subjects, the blood was returned. The auricular pressure rose, but the cardiac output showed no change in the 3 instances in which it was measured. From the above observations it may be concluded: (*1*) Anxiety may cause a 100 per cent increase in cardiac output above the basal state. This may occur without a striking increase in oxygen consumption. (*2*) The auricular pressure may be lowered considerably without affecting the cardiac output and without inducing peripheral vasoconstriction. (*3*) Anxious subjects may maintain an output far above the basal level in spite of a decrease in auricular pressure. The mechanism by which the cardiac output is maintained in spite of a falling auricular pressure has not been determined.

In 3 experiments, primary shock or circulatory collapse occurred shortly after the removal of the blood. In each instance, the subjects complained of epigastric discomfort, nausea, faintness, feeling of heat, and sweating. Consciousness was not lost. They became pale and perspired freely. The heart rate slowed, the mean arterial pressure fell precipitously to approximately one-half of the control value, the auricular pressure did not fall further, and may have risen slightly. The cardiac output was unchanged. Therefore, the calculated total peripheral resistance fell strikingly, indicating arteriolar dilatation. Two of these reactions occurred in one subject. On the first occasion, the patient was anxious and the control cardiac index was 5.1. At the height of the circulatory collapse, the index was 4.9. Four months later, when the experiment was repeated, he was calm and the control cardiac index was 3.4. At the height of the circulatory collapse, it was 3.3. It is to be noted that the signs and symptoms of primary shock appeared at two greatly different levels of cardiac output.

It may be concluded that: (*1*) A fall in cardiac output is not necessary to produce the manifestations of primary shock. (*2*) The fall in arterial pressure is caused by a primary decrease in peripheral resistance rather than by secondary response to a decrease in venous return.

[1] Work done under contract with the Office of Scientific Research and Development.

Experimental Hypertension from Section of Moderator Nerves: The Relationship of the Acute Pressor Response to the Development and Course of Chronic Hypertension.[1] By CAROLINE BEDELL THOMAS, Baltimore, Md.

The moderator nerves of 10 dogs were sectioned at successive operations. Arterial pressures and pulse were recorded by the Hamilton method both with animals unanesthetized and during operations. Inconstant rise and increased lability of pressure first appeared after the third operation, regardless of the order of nerve section. Immediately after cutting the fourth moderator nerve, arterial pressure rose abruptly but soon fell again, and was usually normal a few hours later.

Well marked hypertension appeared 1 to 6 days after the fourth operation, and in some animals it gradually increased in subsequent weeks. Arterial pressures of 425/222 have been recorded. Marked tachycardia and lability of pressure and pulse were found. There was close correlation between heart rate and arterial pressure, indicating the importance of increased cardiac output in this form of hypertension.

Intravenous injection of epinephrin and angiotonin was carried out before and after hypertension had been produced; the increased vasoconstriction produced by the hypertension did not decrease the height of the pressor response to either agent.

Chronic hypertension was observed to persist for 5 years. No pathological lesions attributable to the hypertension were found, with the possible exception of changes in the juxtaglomerular apparatus, which is still under investigation.

Orthostatic Hypotension and Orthostatic Tachycardia in Patients with Diabetic Neuropathy. By R. WAYNE RUNDLES (introduced by Cyrus C. Sturgis), Ann Arbor, Mich.

Observations on 125 patients with diabetic neuropathy revealed orthostatic circulatory abnormalities in 8. Five

[1] Supported by a grant from the Commonwealth Fund for the study of essential hypertension.

patients had attacks of vertigo or syncope when standing, and a fall of 50 mm. Hg or more in the systolic blood pressure when changing from the supine to the erect position. Prompt syncope followed forceful expiration (Flack test). Oliguria and diminished renal function when standing were demonstrated. A young male with a neuropathic joint destruction and a patient with pseudotabes showed smaller postural blood pressure changes but pronounced orthostatic tachycardia. One patient had an average blood pressure when supine of 178/96 (pulse 102) and when standing of 120/80 (pulse 120). Lack of cardiac acceleration was not observed.

These patients all had a common background of several years of poorly managed or neglected diabetes, finally complicated by severe peripheral neuropathy. The majority had, in addition, abnormal pupillary reactions (Argyll Robertson type in 3 cases without evidence of neurosyphilis), chronic or recurrent diarrhea with roentgen evidence of grossly disturbed gastro-intestinal motility, impotence, and atonic bladder paralysis.

Peripheral loss of sweating was shown by sweating tests and vasomotor paralysis by skin temperature studies. Peripheral degeneration of sympathetic nerves appeared to be an important factor in the etiology of the orthostatic circulatory syndrome.

Observations on Renal Venous Blood in Normal Unanesthetized Subjects and Patients with Severe Congestive Heart Failure.[1] By James V. Warren, Arthur J. Merrill, and Emmett S. Brannon (introduced by Charles A. Doan, Columbus, Ohio), Atlanta, Ga.

The present concepts of human renal physiology are based upon somewhat unstable tenets because of the difficulty in obtaining blood from persons immediately before and after its passage through the kidney. The widely used clearance techniques for determining renal blood flow are based upon the assumption that the test substance used is completely removed from the blood during one circulation through the active renal tissue. Arterial blood may be obtained with relative ease from a peripheral vessel because its composition is the same throughout the body, but obtaining venous blood as it leaves the kidney has presented the major problem. The method described here has enabled us to obtain blood directly from the renal vein in the unanesthetized resting subject.

The method is essentially a modification of the technic of right auricular catheterization introduced by Forssman in 1929 and recently extensively utilized by Cournand and his colleagues. A long radiopaque ureteral catheter is passed up the venous system from the antecubital vein into the right auricle and thence via the inferior vena cava to either renal vein. By fluoroscopy, the catheter is seen to pass laterally from the vena cava in the region of the renal pedicle, and to move with the respiratory excursion of the kidney. The position of the catheter may be further checked by intravenous pyelography. Most conclusive evidence that the catheter is in the renal vein is obtained from the comparison of arterial and renal venous blood. The renal venous blood of normal subjects has a high oxygen content and is therefore brighter red than the mixed venous blood from the auricle. As the kidney removes the majority of sodium p-amino hippurate when its concentration in the plasma is low, there is a striking difference in the concentration of this substance in the arterial and renal venous blood following injection of sodium p-amino hippurate into the antecubital vein.

Simultaneous samples of the renal venous and arterial blood were obtained for study of the extraction of oxygen and sodium p-amino hippurate by the kidney. Immediately after obtaining the renal vein sample, the catheter was withdrawn to the auricle and a cardiac output determination was carried out, utilizing the Fick principle.

Observations have been made on patients without apparent renal disease. Several had considerable anemia, as reflected in the oxygen content of the arterial blood. In the 8 cases where it was determined, the renal A-V oxygen difference varied from 1.9 to 2.8 volumes per cent, while in the same subjects the difference in oxygen content between the mixed venous and arterial blood ranged from 3.4 to 6.1 volumes per cent. The cardiac index in these subjects ranged from 2.3 to 3.8. With arterial plasma hippurate levels below 2.5 mgm. per cent, the extraction by the kidney averaged 88 per cent.

Observations were made on 2 patients with chronic congestive failure in whom the cardiac output was decreased. The renal arteriovenous oxygen difference in these patients was 5.5 and 6.5 volumes per cent, suggesting the the renal blood flow was decreased. The renal extraction of hippurate by these patients was 82 and 90 per cent complete during a single circulation through the kidney. Determination of the renal blood flow by the usual clearance technic confirmed the fact that the renal blood flow was greatly decreased.

The ease with which renal venous blood can be obtained opens up a new field of study in patients with kidney disease, hypertension, shock, and cardiac failure.

Plasma Volume and "Extravascular Thiocyanate Space" in Pneumococcus Pneumonia. By David D. Rutstein, K. Jefferson Thomson, Daniel M. Tolmach, William H. Walker, and Robert J. Floody (introduced by L. W. Gorham), Albany, N. Y.

A study of the fluid content of the cardiovascular system and the extracellular space of 46 pneumonia patients was performed.

Plasma volume and "extravascular thiocyanate space" are significantly increased during pneumococcus pneumonia and decrease below normal immediately following recovery. Total blood volume calculated from the plasma volume and venous hematocrit, and total available fluid showed similar changes during pneumonia. Clinically, these patients appeared dehydrated.

[1] Work done under contract with the Office of Scientific Research and Development.

Plasma volume and "extravascular thiocyanate space" of fatal cases are significantly lower than similar measurements made on recovered patients during pneumonia.

Venous hematocrit, red blood cell count, and hemoglobin are unchanged during pneumonia. Therefore, the mean corpuscular volume, the mean corpuscular hemoglobin, and the mean corpuscular hemoglobin concentration are also unchanged during pneumonia.

The findings of this study indicate that the total circulating solutes are diminished less than would be indicated by the measurement of the concentration of such solutes. They also offer an explanation for the precipitation of cardiac failure during pneumonia in patients with heart disease and suggest the need for care in the administration of fluids to cardiac patients suffering from pneumonia.

Non-Hemolytic Streptococci in Primary Atypical Pneumonia.[1,2] By L. Thomas, G. S. Mirick, E. C. Curnen, Jr., J. E. Ziegler, Jr., (by invitation), and F. L. Horsfall, Jr.,[3] New York, N. Y.

A single serological type of non-hemolytic streptococcus was isolated from the lung tissues of 6 of 8 fatal cases and from the sputa of 50 of 91 patients with primary atypical pneumonia. Seventy per cent of 186 patients with this illness developed specific agglutinins against this bacterium during convalescence. Less than 3 per cent of sera from normal persons and patients with other diseases showed similar agglutinins. Specific precipitins against a polysaccharide extracted from this streptococcus appeared during convalescence.

Homologous immune rabbit serum specifically agglutinated this bacterium, gave precipitates with the polysaccharide, and caused capsular swelling. Selected high titer human convalescent sera also gave a positive quellung reaction.

Cultural and biological studies on all strains of this micro-organism isolated yielded strikingly constant results which served to distinguish it from other non-hemolytic streptococci. Antigenically, it was related to but not identical with Type I Streptococcus salivarius (Sherman). It was avirulent for all mammalian species tested but was pathogenic for young chick embryos. The bacterium was resistant to sulfonamides but was susceptible to penicillin.

There is as yet no satisfactory explanation for the results obtained with this micro-organism in primary atypical pneumonia.

[1] The Bureau of Medicine and Surgery of the U. S. Navy does not necessarily undertake to endorse views and opinions expressed in this abstract.

[2] The work described was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and The Rockefeller Institute for Medical Research.

[3] Members of the U. S. Navy Research Unit at the Hospital of The Rockefeller Institute for Medical Research, New York, N. Y.

Pharmacology of Sulfabenamide, a Non-Toxic Fatty Acid Sulfonamide. Prolonged Administration in Cases with Pulmonary Tuberculosis. By Richard Gubner (introduced by J. Hamilton Crawford), Brooklyn, N. Y.

Sulfabenamide differs greatly in its pharmacological behavior from other sulfonamides now in clinical use. The linkage of the aliphatic caproic acid with the amino nitrogen of p-aminobenzenesulfonehydroxamide is stable, resisting hydrolysis *in vitro* and *in vivo,* the compound remaining conjugated in the body to an average of 80 per cent in both blood and urine. Acetylation, accordingly, does not occur except possibly to a negligible degree. Absorption is very rapid, excretion extremely slow. Following a single 5 gram dose, a blood level of 5 to 7.5 mgm. per cent is maintained for several days, falling to half the maximum level after one week. Two-thirds the amount ingested as a single dose is excreted in the urine in one week. Blood levels with dosage 4 to 8 grams daily range between 5 to 10 mgm. per cent, and even with massive dosage, higher blood levels cannot be attained. Urinary concentrations are approximately ten times the blood level.

No toxic effects have been observed when as much as 25 grams were given in a 16-hour period, or in protracted administration to several cases with pulmonary tuberculosis, one patient receiving over 2,000 grams over a 16-month period. Sulfabenamide exhibits the same range of therapeutic activity as sulfanilamide against streptococcal infections, and experimental pneumococcal and *E-typhi* infections. Its lack of toxicity, lipoid solubility, and prolonged retention in the body tissues suggested the possibility of employing the drug in tuberculosis. In 5 cases with advanced bilateral pulmonary tuberculosis, treated with 4 grams of sulfabenamide daily for an average period of 10 months, no deleterious effects of any sort were observed, and an average weight gain of 33 pounds occurred. Concomitant with administration of the drug, sputum became converted to negative in all subjects and roentgenograms showed moderate resolution of pulmonary infiltration. No conclusions are as yet warranted as to the therapeutic efficacy of sulfabenamide in tuberculosis. Preliminary *in vitro* experiments, employing Long's medium, indicate an inhibitory effect on the growth of the tubercle bacillus in concentrations of 5 mgm. per cent and above.

New Preparations for the Treatment of Fungous Infections. In Vitro and In Vivo Experiments with Fatty Acid Salts, Penicillin, and Sodium Sulfathiazole. By Edmund L. Keeney (introduced by Warfield T. Longcope), Baltimore, Md.

Sodium or calcium propionate is incorporated in bread dough and cake batter by many large baking companies to inhibit the growth of molds. In a previous report, we have demonstrated that sodium propionate inhibits the growth of pathogenic fungi and is effective in the treatment of superficial fungous infections.

The fungistatic and fungicidal activities of sodium valerate, sodium caproate, sodium caprylate, sodium caprate, and sodium undecylenate have been determined. These fatty acid salts adjusted to a pH of 7.4 are equal to, or superior to, sodium propionate adjusted to a pH of from 5.5 to 7.4. The relative toxicity of these compounds for albino mice is extremely low.

The clinical effectiveness of a 1 per cent sodium undecylenate ointment and a 10 per cent sodium propionate ointment in the treatment of the superficial fungous infections is comparable. Clinical experiments are in progress on the effectiveness of these salts in higher concentrations.

Two patients with cutaneous actinomycosis have been successfully treated by the daily parenteral administration of a 20 per cent solution of sodium propionate.

Albino mice infected with *Coccidioides immitis* are being treated with the fatty acid salts. The results will be reported.

Penicillin is fungistatic and fungicidal for *Actinomyces bovis*, but fails to possess such activity for the other pathogenic fungi. The same is true for sodium sulfathiazole.

The Cardiovascular Dynamics in Shock Due to Infection. By A. S. Freedberg and H. Haimovici (by invitation), and H. L. Blumgart, Boston, Mass.

Circulatory failure in infectious disease is not well understood. A study of the cardiovascular dynamics has been made in 70 unanesthetized dogs (5 to 14 kgm.) following intravenous injection of a purified toxin obtained from rough colonies of Shiga type dysentery bacilli.

In all animals, injection of this toxin resulted in a state of "shock" associated with a marked fall in cardiac output, increased arterio-venous oxygen difference, decreased stroke volume, lowered central venous pressure, decreased oxygen consumption, fall in blood pressure, slowed circulation time, and tachycardia. The circulating plasma volume showed no change in one-third of the experiments and a variable hemoconcentration in the remainder. The relative blood viscosity paralleled these changes.

The sequence of events is as follows: Within 15 to 30 minutes after the injection of the toxin, a marked decrease in minute volume output from 25 to 75 per cent occurred. This is associated with an increased arterio-venous oxygen difference and a concomitant decrease in right auricular pressure. These early phenomena seem to be related to a decreased venous return. The arterial blood pressure was maintained and evidence of hemoconcentration was absent at first. During the succeeding hours, despite subsequent considerable rises in body temperature, the cardiac output persistently decreased. The blood volume changes noted above became manifest. Later changes in blood pressure may be divided into 3 broad groups: a sudden fall after 1 to 2 hours to 40 to 50 mm.; a maintained blood pressure for many hours with a subsequent progressive decline; and an initial fall with recovery to the control levels, followed by a subsequent decline. The differences between this type of shock and those due to hemorrhage, trauma, and burns are noted.

Bagasse Disease of the Lungs: Clinical and Experimental Aspects. By W. A. Sodeman, and (by invitation) R. L. Pullen, S. S. Pinto, and B. Pearson, New Orleans, La.

Workers breaking bales of bagasse, the product remaining after extraction of sugar from cane, sometimes develop a respiratory illness. Eleven such patients have shown a characteristic clinical picture. Cough and dyspnea occur early, after as little as 3 weeks' exposure. Hemoptysis may be seen. Occasionally, cyanosis is noted. Night sweats, chills, and fever are almost invariably present. On physical examination, chest findings are minimal, but the x-ray discloses an extensive miliary mottling. Sputum is scanty and mucoid and bacteriologic examination does not reveal anything of importance. Leukocytosis develops with a shift to the left and, at times, polycythemia is seen. The entire picture clears up in a period of approximately 3 to 4 months.

Others have suggested fungus infection and allergy to bagasse as the cause. In a series of skin tests with various bagasse extracts we have found reactions in controls as well as in patients exposed to bagasse. Lung biopsy from one of our patients has shown bagasse particles in the lung tissue, indicating a type of pneumoconiosis. The lung reaction is a peculiar one which has been studied further by Miller's technic, consisting of the injection of extracts into the peritoneal cavity of the guinea pig. Various fractions of bagasse have been injected to determine the reaction to various chemical fractions, with results indicating the presence of a toxic material in bagasse, the relative unimportance of the silica fraction, and tissue responses of a transient nature, indicating a reversible reaction as seen in the clinical picture of bagasse disease.

Control of Hyperthyroidism with Thiouracil and Continued Remission After Therapy. By E. B. Astwood, Boston, Mass.

Thiouracil (0.2 to 0.6 gram daily) was administered to 11 normal and 51 hyperthyroid persons. In 33 cases of primary hyperthyroidism, the basal metabolic rate began to fall in 6 (3 to 18) days and was normal in 23 (11 to 46) days. Slower responses were noted in 8 cases which had received iodine, 8 cases of toxic nodular goiter, and 2 cases of acromegaly. The normal individuals were treated for 6 weeks without effect. The latent period was apparently related to the quantity of stored hormone and the rate of its release.

A subsequent maintenance dose of 0.1 to 0.2 gram daily controlled all symptoms and maintained normal metabolic rates. Exophthalmos usually regressed slowly and no instance of malignant progression occurred. Some cases with large diffuse goiter experienced a further enlargement and accentuation of the bruit which later subsided.

Added iodine in 2 such cases induced a further fall in the metabolic rate and disappearance of the bruit. Four cases exhibited a febrile reaction on the 9th to 11th day.

Treatment was discontinued after 6 to 8 months in 9 cases; in each, the metabolic rate remained normal and to date, 2 to 8 months later, there has been no evidence of a return of the disease.

Serum Iodine and Basal Metabolism of Myxedematous and Euthyroid Subjects Treated with Desiccated Thyroid. By DOUGLAS S. RIGGS, (by invitation), EVELYN B. MAN, (by invitation), and ALEXANDER W. WINKLER, New Haven, Conn.

Serum iodine values and basal metabolic rates in 10 myxedematous and in 10 euthyroid subjects have been followed at various levels of thyroid medication. In myxedema, serum iodine and metabolic rate varied directly with the thyroid dose within the limits of zero and 2 grains daily. In contrast, in euthyroid subjects receiving larger doses, the response was much smaller and more irregular, so that frequently an increase in dosage produced no response. Insofar as a response developed, however, the increase in metabolic rate for a given increase in serum iodine was the same in both groups.

In euthyroid subjects, cessation of thyroid medication frequently resulted in transitory subnormal levels of serum iodine without comparable depression of metabolism, suggesting temporary inhibition of normal thyroid activity. Tolerance of euthyroid subjects to amounts of thyroid far in excess of normal production of hormone implies, in addition, inactivation of a large part of the exogenous hormone. Breakdown of exogenous hormonal iodine to inorganic iodine by the thyroid gland itself is suggested.

*The Effect of Thyrotropic Hormone in the Collection of Radio-Iodine, Mean Acinar Cell Height, and Gland Weight in the Thyroid of the Chick.** By F. R. KEATING, JR., RULON W. RAWSON, *and* WENDELL PEACOCK (introduced by J. H. Means), Boston, Mass.

Chicks were injected with radio-active iodine having a half life of 8 days and the quantity collected by the thyroid was measured with a Geiger counter. Mean acinar cell height and thyroid weight were determined in other chicks similarly treated. With increasing degrees of stimulation by thyrotropic hormone, there is a progressive increase in collection of radio-iodine by the thyroid until a plateau is reached. This increase parallels that in mean acinar cell height. When the plateau in collection of radio-iodine is reached, thyroid weights have only begun to increase. When the effects of thyrotropic hormone are plotted against time, the mean acinar cell height increases promptly and definitely in less than 24 hours while the collection of radio-iodine and thyroid weight lag behind about forty-eight hours. The collection of radio-iodine and mean acinar cell height later level off but thyroid weights continue to increase. The collection of radio-iodine by the thyroid thus appears to be proportional to the degree of cellular hypertrophy. An increase in the collection of radio-iodine occurs only after hypertrophy has taken place.

* From the Institute of Experimental Medicine, The Mayo Foundation Graduate School, University of Minnesota, Rochester, Minnesota (Dr. Keating); the Thyroid Laboratory, Massachusetts General Hospital, Boston, Massachusetts (Dr. Rawson); and the Nuclear Physics Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts (Dr. Peacock).

Influence of Fat in Diet on Cholesterol Ester Fatty Acids in Hypothyroid State. By ARILD E. HANSEN, Galveston, Tex.

In none of the studies reporting increased values for the serum cholesterol and cholesterol esters in hypothyroidism has the character of the fatty acids involved been investigated. In a detailed study of the serum fatty acids in 3 children with hypothyroidism, we found those in the acetone soluble fraction (in which the cholesterol esters are present) to have higher iodine numbers than did those in the acetone insoluble fraction. This indicated that the fatty acids present as cholesterol esters were highly unsaturated, which was actually demonstrated in one case by enzymatic hydrolysis.

In order to determine whether or not this elevation of the cholesterol esters in hypothyroidism might be dependent upon the availability of highly unsaturated fatty acids (which cannot be synthesized by animals) in the diet, detailed determinations were undertaken of the serum fatty acids in 2 mongrel dogs, one receiving 28 per cent of his calories as fresh lard, the other but 0.16 per cent of his diet as fat. Following thyroidectomy, in the animal receiving fat, there was a distinct rise in the cholesterol esters. The acetone soluble fatty acids were greater in quantity, and their iodine numbers remained relatively high, indicating that, as in human subjects, the cholesterol ester fatty acids involved were highly unsaturated. In the animal on the diet almost devoid of fat, the cholesterol esters actually decreased, and there was no increase in either the total amount or degree of unsaturation of the acetone soluble fraction.

These preliminary findings suggest that the increase in cholesterol ester fatty acids in the hypothyroid state is dependent upon the availability of unsaturated fatty acids in the diet.

Alloxan Diabetes in Rabbits and Rats. By C. CABELL BAILEY and ORVILLE T. BAILEY (introduced by Howard F. Root), Boston, Mass.

Previous observations showed that injections of alloxan into rabbits caused necrosis of the islets of Langerhans and fatal hypoglycemia.

Believing diabetes inevitable under such conditions if hypoglycemic death could be prevented, 2 rabbits were

given 200 mgm. of alloxan per kgm. of body weight, and protected with concentrated glucose intravenously. Within 36 hours, persistent severe diabetes was present.

Histologic sections, 3 days after diabetes developed, revealed selective necrosis of the pancreatic islets of Langerhans without injury to acini and with only minor changes in other organs. In 2 additional rabbits, killed immediately after diabetes developed, microscopic sections showed some islet cells with hydropic degeneration, others with irreversible changes, some essentially normal, while occasional cells were in the process of mitotic division.

Diabetes in rats is best produced by the subcutaneous injection of 200 mgm. of alloxan per kgm. The intravenous and intraperitoneal routes did not prove feasible. Eighteen rabbits and 22 rats with alloxan diabetes have been studied in metabolism cages.

Diabetic cataracts developed in 6 weeks in alloxan diabetic rabbits kept alive with insulin and in 3 months in diabetic rats.

Diabetic acidosis and coma have been produced in both alloxan rabbits and rats.

Some Metabolic Aspects of Damage and Convalescence. By J. S. L. Browne, Victor Schenker, and J. A. F. Stevenson, Montreal, Canada.

In healthy adult men after burns, fractures, wounds, operations, etc., there occurs a negative nitrogen balance. This lasts for from 5 to 45 days in the cases studied, depending on the nature and severity of the trauma and on the individual reaction of the patient. At the height of this period of negative nitrogen balance, oral intake of protein up to about 100 grams (2000 cals.) per day is followed on the same day by the excretion of practically the entire nitrogen content of the protein as urea in the urine. The nitrogen of intravenously administered plasma does not appear in the urine in this manner. Preliminary experiments indicate that the nitrogen of intravenously administered protein hydrolysate (Amigen) behaves as does food nitrogen.

The period of negative nitrogen balance is succeeded by one of positive balance. Intercurrent damage such as upper respiratory infection, any increase in fever, etc., is accompanied by an increase in nitrogen output even though the intake may not be altered. This leads to increased negative nitrogen balance. Mild exercise, such as wheeling around in a wheel chair for the first time, or other fatiguing procedures, such as changing a cast, leads during the early part of convalescence to an increased nitrogen output on the same day and to a decrease in positive nitrogen balance or even to a negative balance.

Persons chronically ill before operation also show negative nitrogen balance after operation but differ from healthy adults acutely damaged in that raising the protein intake to moderate levels does not lead to the immediate excretion of all the food nitrogen. These patients can thus be brought into positive nitrogen balance at an earlier period and at a lower level of intake.

Metabolic Studies on the Urinary Excretion of Ketosteroids in Normal Individuals and in Patients with Adrenal Dysfunction and Cancer. By C. P. Rhoads and (by invitation) Konrad Dobriner, New York 21, N. Y., and Seymour Lieberman, B. R. Hill, and Louis F. Fieser, Cambridge 38, Massachusetts.

The determination of the 17-ketosteroid levels in the urines of normal individuals and of patients does not allow for a distinction between the different kinds of steroids excreted nor does it permit the quantitative estimation of the amount of each compound present, both of which may be significant for certain disorders.

Individual urine collections were made and the steroid content studied in a group of normal men and women of different ages, in pregnancy, in patients with adrenal dysfunctions (adrenal hyperplasia, cortical tumor and Cushing's syndrome), and in patients with cancer and leukemia. The neutral fractions obtained from these urines were separated into the ketonic and non-ketonic material. The ketonic components were further divided into their alpha and beta ketonic fractions. The non-ketonic material was separated into its alcoholic and non-alcoholic constituents.

In all, 33 different ketosteroids were isolated by systematic quantitative chromatographic analysis of the alpha ketosteroids from the individual urines. Seven of these have been previously described in the literature. Only 9 alpha ketosteroids are constantly found in the urine of normal men and women. Their distribution also follows a consistent pattern. About 60 to 70 per cent of the alpha ketosteroid fraction consists of androsterone and its isomer, alpha hydroxyaetiocholanolone which are present in a ratio of about 1:1. The urines of pregnant women and of patients with adrenal hyperplasia and adrenal cortical tumors reveal not only the presence of a great number of ketosteroids not found in normal urine, but also a distinct difference in the pattern of their distribution. In the urine of certain cancer patients, ketosteroids are present which are not found in the urine of normal persons. In others, androsterone is absent or an abnormal ratio of androsterone to aetiocholanolone is found.

The study of the alpha ketosteroid patterns in normal and diseased individuals reveals marked differences in the distribution and types of compounds present. These results give more detailed information with regard to the disturbances of steroid metabolism.

*Clinical Actions of Ethylnorsuprarenin.** By M. L. Tainter, W. M. Cameron, L. J. Whitsell, and M. M. Hartman (introduced by T. G. Klumpp), New York, N. Y.

The chemical compound 1-(3-4 dihydroxyphenyl)-2-amino-1-butanol hydrochloride or ethylnorsuprarenin is

* Supported, in part, by Therapeutic Research Grant 437 of the Council on Pharmacy and Chemistry, American

a sympathomimetic amine, closely resembling epinephrine in many of its actions. However, it lacks the power to raise systolic blood pressure like epinephrine, but rather lowers the diastolic pressure and increases the pulse rate. Therefore, it causes an increase in pulse pressure, and presumably in the volume flow of blood, without a proportionate rise in cardiac work.

In animals, ethylnorsuprarenin is approximately 1/120 as toxic as epinephrine for fatal effects intravenously, and does not cause excitation of the central nervous system.

In patients, ethylnorsuprarenin may be injected subcutaneously, intramuscularly, or intravenously in doses of from 0.5 to 2.0 mgm., with typical effects which appear in from 1 to 5 minutes and persist for from 20 minutes to an hour, depending on the dose and route of administration.

It is effective in the relief of acute asthmatic attacks, giving relief equal to that of epinephrine in doses about one-half larger. This relief is accompanied by fewer and less marked subjective side effects, such a precordial pain, nausea and vomiting, excitation, etc., than might be experienced by the same subjects after equivalent doses of epinephrine.

Accordingly, ethylnorsuprarenin would appear to be useful in the treatment of asthma, and possibly preferable to epinephrine for those patients in whom epinephrine administration is accompanied by undesirable side-actions. Further clinical trials appear to be justified along these and other lines.

Uric Acid Metabolism Studies in Gout. By R. H. Freyberg and (by invitation) W. D. Block and N. Geib, Ann Arbor, Mich.

The effects of different diets, the oral administration of various purines, beef extract, cincophen, sodium salicylate, colchicine, and the intravenous injection of lithium urate were studied in a patient with tophacious gout and in a normal control subject. The amount of uric acid in the blood and 24-hourly urine collections was measured by a recently perfected specific and highly accurate uricase method, as well as the usual silver precipitation, and direct methods, and the results were compared. The silver isolation procedure gave slightly higher values for "uric acid" than did the specific uricase method; but usually it was fairly accurate.

In the gouty patient and control subject, the results were similar. The ingestion of different amounts of purine-free protein, xanthine, uric acid, arginine, and histidine did not significantly change the blood or urine uric acid values. About 25 per cent of the injected lithium urate was accounted for by increase in excretion of uric acid. When caffeine was ingested, the non-specific methods showed a great increase in "uric acid", but the specific uricase method showed only slight increase in urine true uric acid with a large increase in other substances giving the color reaction. Ingestion of beef extract caused a substantial increase in the uric acid of blood and urine. Ingestion of adenine sulfate caused an increase in blood and urine uric acid and other substances giving the color reaction—probably other purine degradation products. Guanine caused similar changes but to lesser degree. Cincophen caused considerable increase in the excretion of uric acid and lowered blood uric acid. Sodium salicylates caused an increase in uric acid and in non-uric acid chromogenic substances.

The results of these studies are discussed in regard to reliability of various analytical methods, and in regard to the problem of the metabolic defect characterizing gout.

*The Function of the Sweat Glands in the Economy of NaCl Under Conditions of Hard Work in a Tropical Climate.** By Jerome W. Conn and (by invitation) Margaret W. Johnston, Ann Arbor, Mich.

Acclimatized men performing hard work (4000 to 4200 cals per 24 hours) in humid heat (85° F. and 85 per cent humidity) and producing 4 to 9 liters of sweat per 24 hours, remain in NaCl balance and retain physical 'fitness' on a total dietary intake of NaCl as low as 5 grams per day. This is accomplished by the ability of the sweat glands to produce a fluid containing progressively lower concentrations of NaCl as the supply of salt in the diet is diminished and as urinary NaCl falls off sharply (indicating a need to conserve body salt).

With work, sweat volume, and sweat NaCl concentration constant, an abrupt change from high to low NaCl intake produces the following sequence of events: (*1*) A marked fall in urinary NaCl, (*2*) followed in 1 to 2 days by a significant fall in sweat NaCl concentration, (*3*) a gradual rise in urinary NaCl until urine plus sweat losses approximate total NaCl intake, (*4*) continuation of the newly acquired level of more dilute sweat.

When, under the same conditions of low salt intake and continued existence in humid heat, work periods are discontinued for several days (abruptly lowering total sweat volume), there occurs (*1*) a rapid rise in urinary NaCl, (*2*) followed by an increase of sweat NaCl concentration. If increased sweating is now produced by heavy clothing without work, urinary NaCl falls followed by a decrease in sweat NaCl concentration.

Under conditions of these experiments, an average diet, containing 10 to 15 grams of NaCl, provides sufficient protection in acclimatized men to make the use of salt supplements unnecessary.

Medical Association, and by grants from the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

* Work done under contract with the Office of Scientific Research and Development.

*Studies of Work and Discomfort in Patients with Neurocirculatory Asthenia.** By MANDEL E. COHEN (by invitation), ROBERT E. JOHNSON (by invitation), STANLEY COBB, WILLIAM P. CHAPMAN (by invitation), and PAUL D. WHITE, Boston, Mass.

The type of patients studied were men who have complained for many years of dizziness, palpitation, chest discomfort, and dyspnea. This has been associated with inability to do hard work, nervousness, and emotional instability. Control subjects were healthy soldiers.

In studies of ability to do hard work (run on the treadmill at 7 miles per hour, 8.6 degrees grade) the patients (43), when asked to run to exhaustion, ran a much shorter time (mean 79.6 seconds) than did 59 normal control subjects (mean 201.6 seconds). Step tests (20 inch step, 30 steps per minute) in 51 patients gave mean time of 79.1 seconds, compared to mean of 190.3 seconds in 40 control subjects. Lactic acid determinations taken five minutes after end of run on the treadmill revealed a lower level (32 patients, mean 77.1 mgm. per ml. blood) than the normal controls (41 control subjects, mean of 122.7 mgm. per 100 ml. of blood).

Studies of moderate work (walk on the treadmill at 3.5 miles per hour, 8.6 degrees grade) allow patients to finish the task. Lactic acid determinations taken at the 10th minute of walking yield results almost twice as high in 26 patients compared with 40 normal control subjects (mean in patients, 36.4; in controls, 21.6 mgm. per 100 ml. blood). These results are comparable with similar studies in poorly trained men and in women.

An attempt was made to train a selected group of 23 patients with neurocirculatory asthenia under the direction of an athletic trainer, experienced in this type of training. Half the program involved methods of kindly persuasions; and the other half coercive methods applied by an Army sergeant. Neither method was successful. The subjects were unable or unwilling to undergo the type of training necessary for what the trainer called "real training," *i.e.*, pushing past limits of comfort. The patients complained that uncomfortable symptoms prevented their making the necessary effort.

Studies were made to test the patients' reaction to uncomfortable stimuli which were not particularly related to the cardiovascular apparatus. Reaction to pain was tested on a modification of the Hardy-Wolff heat radiation apparatus. Levels of perception of pain were approximately equal. However, 39 patients reacted to pain with a lower stimulus—0.315 gram cl. per sec. per cm. sq. as compared with 47 control subjects who reacted at 0.353 gram cl. per sec. per cm. sq. Ability to stand uncomfortable electric shock was lower in patients; 29 patients standing a mean of 2.5 units as against 50 control subjects standing a mean of 6 units. Units were arbitrary units of electrical current and are reported as reaction level minus awareness level. In testing ability to maintain grip at 60 per cent of maximum using a Stoelting hand dynamometer 22 patients maintained a constant grip for 31 seconds. Fifty-six control subjects maintained a constant grip for 48 seconds. It was found that the patients did poorly on types of tests other than those which involve cardiovascular respiratory function.

It was concluded from this study that patients' disability was not limited to the cardiovascular-respiratory system alone.

* The work described in this paper was done under a contract of the Office of Scientific Research and Development, Contract OEM cmr 157, with the Massachusetts General Hospital, Boston, Mass.

Clinical, Chemical, and Physicochemical Studies on Human Plasma Preserved in the Liquid State at Room Temperature. By EUGENE L. LOZNER and (by invitation) LLOYD R. NEWHOUSER, Bethesda, Md.

The preservation of normal human plasma in the liquid state at room temperature for periods up to 2 years has been evaluated by an analysis of administrations and by chemical and physicochemical studies. Over 1300 transfusions of liquid plasma more than a year old have been performed with an untoward reaction rate actually less than that of fresh or dried plasma. The plasma had lost none of its therapeutic effectiveness as an anti-shock or anti-hypoproteinemia agent as judged by clinical response, increase in blood pressure, decrease in pulse, and increase in plasma proteins.

The amino acid nitrogen and polypeptide nitrogen content increased slightly during the second year of liquid storage. The actual values indicate hydrolysis to this degree of less than 2 per cent of the original protein. The electrophoretic pattern of liquid plasma 2 years old shows the mobilities of the globulin factors to be nearer and invading somewhat the albumin component. Stored plasma shows a slightly greater osmotic effectiveness than fresh plasma *in vitro*. Neither the chemical nor the physicochemical changes are of sufficient magnitude to preclude the use, for its colloid content, of properly prepared liquid plasma stored up to at least 2 years.

The Mechanism of Pain in Aviators' "Bends". By JOSEPH P. WEBB, GEORGE L. ENGEL, JOHN ROMANO, HENRY W. RYDER, CHARLES D. STEVENS (by invitation), M. A. BLANKENHORN, and EUGENE B. FERRIS, JR., Cincinnati, Ohio.

Because of the effect of denitrogenation (preflight oxygen inhalation) in preventing decompression sickness, there has been general agreement that the symptoms are caused by the appearance of bubbles of gas, chiefly nitrogen, incident to decompression. Moreover, the view has been widely held that the pain of "bends" is based on tissue ischemia secondary to vascular occlusion by bubbles; however, the following evidence suggests that distortion of tissues by extravascular bubbles is the more likely explanation.

(*1*) Nitrogen clearance studies indicate that bubble formation would be expected to occur first in tissues having the poorest blood supply, and last in arterial blood.

(2) It has not proved possible to demonstrate bubbles in venous blood during "bends".

(3) The application of external pressure to a painful area relieves the pain, despite the fact that the pressure may oblierate blood flow.

(4) The specific pain pattern may be reproduced exactly by re-exposure after intervals as long as 6 hours, indicating that the circulation has not removed the bubble.

(5) The vascular phenomena which sometimes complicate decompression sickness (skin and neurologic manifestations) have been demonstrated to be of spastic rather than of embolic nature.

(6) Finally, roentgenographic studies of the knee joint show a highly significant correlation between the presence of pain in the region of the joint and the presence of extra-articular tissue bubbles which have a non-vascular distribution.

READ BY TITLE

*The Mechanism of Shock from Burns and Trauma Traced With Radiosodium.** By CHARLES L. FOX JR., and ALBERT S. KESTON (introduced by A. R. Dochez), New York, N. Y.

Investigations by Rosenthal in mice and by Fox in man have demonstrated the therapeutic efficacy of isotonic solutions of sodium salts in shock and have shown the occurrence of a large sodium deficiency. To explore further the relative shifts of sodium and water in normal and traumatized tissues, radiosodium was used.

Shock from both burns and trauma was standardized in mice by Rosenthal's technics. Isotonic saline containing Na^{24} was given before injury so that body sodium was "tagged" and in other animals was administered therapeutically during shock. Determination of Na^{24} and water content was performed in normal and injured tissues and in control animals.

The results show that in traumatized tissues: (1) the water content increased up to 10 per cent, whereas the sodium content increased from 300 to 500 per cent; (2) the volume of extracellular fluid was greatly increased; often all the tissue water appeared to contain sodium, indicating entrance of sodium into cells normally devoid of it. The extracellular fluid of uninjured organs was reduced. The total volume of extracellular fluid was decreased more than 50 per cent; in severe human burns, similar reductions were found.

The data indicate a great "loss" of sodium into traumatized tissues, accompanied by a marked reduction in the total volume of extracellular fluid. The circulatory collapse characteristic of shock, and the usually concomitant decrease in plasma volume, are apparently the consequence of the sharp reduction in extracellular fluid volume. Accordingly, the primary therapeutic indication is rapid restoration to normal of the extracellular fluid volume, most efficiently accomplished by administration of solutions which have the effect of rapidly restoring to normal concentration the chief extracellular component, namely sodium.

* Work done under a contract with the Office of Scientific Research and Development.

The Effect of Vitamin K_1 Oxide upon the Anticoagulant Action of Dicoumarol. By CHARLES S. DAVIDSON, JOHN H. FREED, and HARRIET MACDONALD (introduced by Clark W. Heath), Boston, Mass.

The usefulness of dicoumarol, in contrast to the rapid action of heparin, in patients in whom delayed coagulability of the blood is desired, is limited by the time required both for delayed blood coagulability to occur and for normal coagulability to return after discontinuing the administration of dicoumarol. Vitamin K-like substances and vitamin K_1 oxide have been shown to have an action antagonistic to that of small amounts of dicoumarol on the blood prothrombin concentration. The present study concerns the effect of vitamin K_1 oxide upon the action of dicoumarol given in doses sufficiently large and over a sufficiently long time not only to reduce the blood prothrombin concentration but also to prolong the blood coagulation time significantly.

Five patients in whom delayed blood coagulability was desired were given dicoumarol orally. The initial dose was 0.8 gram, followed by 0.2 gram daily. Administration was continued until the venous blood coagulation time, as measured by a modification of the method of Lee and White, was 15 minutes or longer. This required a total dose of from 1.0 to 2.0 grams given over 3 to 8 days. At this time, vitamin K_1 oxide was given as a single intravenous dose of 0.5 or 1.0 gram. The coagulation time then always returned to normal (6 to 12 minutes) in from 3½ to 10 hours. In 4 of the patients, this occurred in spite of the continued administration of the maintenance dose of dicoumarol. In 3 of the 4, no increase in blood coagulation time again occurred in from 4 to 16 days. In the fourth patient, whose illness was complicated by obstructive jaundice, a prolonged coagulation time re-appeared 24 hours after the administration of the vitamin K_1 oxide.

The administration of vitamin K_1 oxide, as here reported, is, so far as is known, the first effective antagonism of the anticoagulant action of dicoumarol. The action of vitamin K_1 oxide is, however, slow compared to the rapidity with which the coagulation time of the blood returns to normal after cessation of the administration of heparin. Moreover, after an effective dose of vitamin K_1 oxide, the administration of dicoumarol will not again prolong the coagulation time until from 4 to 16 days later. By contrast, resumption of intravenous heparin has a prompt action. Thus, dangers and inconveniences still attend the use of an anticoagulant as difficult to control as is dicoumarol.

The Absorption and Excretion of Penicillin Following Continuous Intravenous and Subcutaneous Administration. By LOWELL A. RANTZ and (by invitation) W. M. M. KIRBY, San Francisco, Calif.

The absorption and excretion of penicillin during continuous intravenous administration has been studied in a

group of syphilitic individuals under basal conditions. The concentration of penicillin obtained in the plasma was proportional to the amount administered Twenty-five hundred units per hour induced a concentration of 0.05 unit per ml.; 5,000 units per hour, 0.10 unit per ml.; 10,000 units per hour, 0.2 unit per ml.; and 20,000 units per hour, 0.4 unit per ml.

Seven hundred and fifty to 1,000 ml. of plasma per minute were cleared of penicillin by the kidney, a rate which indicates that the renal tubule is primarily involved in the excretion of this substance. Infusion of penicillin in quantities up to 20,000 units per hour failed to establish a maximum rate of tubular excretion (Tm). Excretion is quantitative and the rate is unaffected by the minute volume of urine.

The plasma concentrations of penicillin were determined during its routine administration to sick individuals and were found to approximate those obtained during controlled study if the intravenous route were utilized. Subcutaneous infusion resulted in levels only 50 per cent as great.

Effect of Sulfonamide Chemotherapy upon Experimental Bacterial Pneumonia in the Presence of Influenza Virus. By CARL G. HARFORD (by invitation), MARY RUTH SMITH (by invitation), CHARLOTTE McLEOD (by invitation), and W. BARRY WOOD, JR., St. Louis, Mo.

Most of the deaths occurring in the influenza pandemic of 1918 are thought to have been due to secondary bacterial pneumonia. Although it has been assumed by many that such bacterial infections can be controlled by sulfonamide chemotherapy, no proof has been offered that the sulfonamide drugs are effective in the presence of influenza virus.

This problem has been studied experimentally in mice infected with Type A influenza virus and either Type I pneumococci or hemolytic streptococci. Experiments have been carried out using both lethal and sublethal doses of virus together with doses of bacteria which produce fatal pneumonia in the absence of virus. The infection has been initiated by intrabronchial inoculation of virus and bacteria. If untreated, animals thus infected die within 48 hours of a fulminating bacterial infection. The treated animals survive the bacterial infection, and when a lethal dose of virus is employed, they die between the fifth and ninth days. If sacrificed on the fifth day, their lungs contain no bacteria.

It was also shown that a mild pulmonary infection can be produced in rats by intrabronchial inoculation of influenza virus. Pneumococcal pneumonia superimposed upon the virus lesion was found to respond to chemotherapy.

It is concluded from these studies that sulfonamide drugs are effective in the treatment of experimental bacterial pneumonia, even in the presence of a lethal dose of Type A influenza virus.

The Effect of Testosterone and Thiouracil on the Metabolic Disturbances of Progeria. By N. B. TALBOT, A. M. BUTLER, and E. A. MACLACHLAN (by invitation), Boston, Mass.

Prolonged metabolic studies on a 6-year-old, dwarfed, cachetic boy with typical progeria showed that:

1. The food he assimilated was adequate for rapid growth in a normal child, yet he had not grown for several years.
2. Weight gain, nitrogen deposition, and body growth were induced by testosterone therapy. This showed that the patient *could* grow.
3. However, it was then noted that: (*a*) testosterone gradually lost its effectiveness; (*b*) he should have gained more weight (muscle) according to nitrogen, potassium, sodium, chloride, calcium, and phosphorous balance measurements than he actually did; and, (*c*) he had a very high energy output. This suggested that he might be gaining muscle at the expense of body fat or carbohydrate required for energy production.
4. To reduce the energy metabolism governed by the thyroid, thiouracil was given in addition to testosterone. This resulted in a reduction in energy output (BMR, insensible weight loss) and a simultaneous and striking gain in weight with a corresponding nitrogen storage. During this course of therapy, the patient changed from a thin, hyperactive individual to a taller and more plump and normally placid person.

It is concluded that progeria is associated with marked metabolic disturbances, which can be modified by a specific therapy.

*A Study of Proteolytic Enzymes in Lymph.** By PAUL C. ZAMECNIK and JOHN B. GRAHAM (introduced by Oliver Cope), Boston, Mass.

Recent studies on experimental burns have called attention to the zone of partially damaged cells which surrounds the severely burned area as a possible site of origin of toxins. In the present investigation, a search for proteolytic enzymes was made in lymph from dogs' legs, before and after burning the legs by immersion into water at 90° C. for 15 seconds.

With the aid of peptide substrates, the presence of an aminopeptidase was detected, hitherto undescribed in lymph. In the lymph draining the burned area, the activity of this enzyme rose from 6- to 20-fold within an hour after the burn, and continued to remain elevated for a period of hours thereafter. The known characteristics of this enzyme are as follows: it has a pH optimum around 7.5, hydrolyzes 1-leucylglycylglycine preferentially, and does not require the presence of any known activator.

Following a crushing injury to the dog leg, this enzymatic activity was found at increased levels in the efferent

* The work described here was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Massachusetts General Hospital.

lymph from this area. An enzyme with properties so far identical to those described has been found in saline extracts of normal dog muscle. The same type of enzymatic activity was found in bleb fluid obtained from human burns.

A Limited Study of the Recent Influenza Epidemic. By MAXWELL FINLAND and (by invitation) MILDRED W. BARNES and BERNARDO A. SAMPER, Boston, Mass.

Isolation of virus from acute cases of influenza was accomplished by inoculation of pharyngeal washings intranasally in mice and into the allantoic sac of the developing chick embryo. Evidence of the successful isolation was obtained in the first egg passage and in the third to the sixth mouse passages. All the viruses isolated from patients during the month of December and the early part of January were identified as influenza A. High titers of complement-fixing antibodies for the laboratory-adapted PR-8 strain of influenza A developed during convalescence in all of the patients from whose pharyngeal washings the virus was isolated. A rise in antibodies was also demonstrated in similar cases of clinical influenza from which isolation of virus was not attempted. In addition, a similar virus was obtained during the height of the epidemic, from the lung of an acutely fatal case of pneumonia which followed influenza.

After the end of January, cases of clinical influenza were again observed and virus was readily isolated from pharyngeal washings of acute cases and from the lungs of fatal cases. Evidence has been obtained which suggests that this may be a different virus or a variant.

Serological studies were made in a number of severe cases of bacterial pneumonia, observed shortly after the height of the epidemic. Many of these patients responded poorly to the usual treatment. In the serum of most of these patients, significant and sometimes high titers of antibodies for influenza A were demonstrated.

*Some Effects of the Administration of Amino Acids in a Patient with Idiopathic Steatorrhea.** By KENDALL EMERSON, JR. and WILLIAM W. BECKMAN (introduced by T. M. Rivers), New York, N. Y.

Amino acids in the form of an enzymatic hydrolysate of casein were administered to a 27-year-old woman with idiopathic steatorrhea, hypoproteinemia, and hypocalcemia. After a control period on a measured diet, 40 grams of the daily dietary protein were replaced by an equal amount of the casein hydrolysate as a 10 per cent solution given intravenously in 3 daily portions for one week. During the control period, the patient was in negative nitrogen and phosphorus balance. Two days after the commencement of intravenous amino acids, the diarrhea ceased and the stools became normal. During the week of treatment, there was a marked retention of nitrogen, averaging 3 grams daily; both fecal and urinary nitrogen d creased. No change in calcium or phosphorus excreti occurred. Following cessation of therapy, the diarrh returned within 4 days. The patient was then given grams of the amino acid mixture by mouth daily for o week, in addition to the control diet. Again the stoo promptly returned to normal, and a marked nitrog retention occurred. This time there was a definite i crease in the retention of calcium and phosphorus.

* The Bureau of Medicine and Surgery of the U. S. Navy does not necessarily undertake to endorse views and opinions expressed in this abstract.

Thiamine Metabolism and Excretion in Man. B BENJAMIN ALEXANDER, GRETA LANDWEHR, a FRANCES MITCHELL (introduced by Mark D. Al schule), Boston, Mass.

The exact relationship between the amount of admi istered thiamine and its urinary excretion requir elucidation. Furthermore, a large proportion of a ministered thiamine cannot be recovered in the excret

In 3 normal subjects, intramuscularly injected thiami was increased daily by both geometric and arithmet proportion. Experiments by different quantitative tec nics gave the same excretion curve. Thiamine determin tions were made by the specific colorimetric meth developed here. In one subject, analyses of urina pyrimidine were also made.

Urinary thiamine was closely related to administer thiamine, rose with increasing daily doses, was 50 p cent at the 10 mgm. dose, and 80 to 90 per cent at t 35 mgm. dose.

All excretion curves differed only slightly. Oth isolated observations here and in the literature were good accord.

One subject received 1.086 grams of thiamine in days; 82 per cent was recovered as urinary thiami and 16 per cent as pyrimidine; 0.5 mgm. per day w unaccounted for. A 9-day balance study, beginning lon after discontinuation of parenteral injection, gave the sa value. Since fecal thiamine is of this magnitude a since some thiamine appears in sweat, probably all t thiamine taken is excreted as thiamine or pyrimidine.

The amount of thiamine which can be converted in pyrimidine was measured during the period of hig thiamine dosage. The subjects differed in age, weigh metabolism, and surface area, and represented both sexe Maximal pyrimidine conversion was proportional to t oxygen consumption per unit of surface area.

*Diffusion of Water Through Living and Dead Huma Skin with the Identification of the Diffusion Inhibitin Layer.** By GEORGE E. BURCH and (by invitation TRAVIS WINSOR, New Orleans, La.

The rate of water loss from the surface of the skin o living and dead human subjects was measured quantita tively. It was found that the rate of water loss throug dead skin studied within 2 hours of death, 4 weeks afte death, or after repeated freezing and thawing was essen

* Aided by a grant from the Rockefeller Foundatio

tially the same and of the same order as the loss through intact skin of living subjects resting quietly in a comfortable environment (temperature 75° ± 1° F.; relative humidity 50 per cent ± 2 per cent). After an interval as long as 60 minutes, no water accumulated in the collecting chambers sealed to the intact living skin of the comfortable resting subject, indicating the absence of sweat gland activity. The rate of water loss through the skin of 2 patients with atrophy of sweat glands who were unable to sweat and who suffered from summer heat was the same as that through dead skin or the intact skin of resting comfortable subjects. It was concluded that water lost through the skin of normal subjects resting quietly in a comfortable environment is lost by diffusion and not through sweating by the sweat glands. The rate of diffusion was found to vary directly with the temperature of the skin. The rate of diffusion of water through human skin was essentially the same as through the "skins" of fruits and vegetables.

By isolating the layers of the intact and dead human skin by means of cantharides plasters, mechanical methods, and fat solvents to remove the superficial fats and oils, and checking the completeness of removal by tissue sections, the rate of diffusion through the various layers of the skin was determined. It was found that the thin dead superficial layer, the corneum, was the main layer responsible for the inhibition of diffusion through human skin.

*Factors Which Influence the Number of Hemolytic Streptococci in the Air of Hospital Wards.** By MORTON HAMBURGER, JR. (introduced by Dr. C. Philip Miller), Camp Carson, Colo.

Studies of the distribution of hemolytic streptococci in the environment of patients harboring these microorganisms in the nose and throat show that they accumulate rapidly in bedclothing and floor dust. Cultures of bedlinen made as soon as 4 hours after admission of a patient sometimes revealed thousands of hemolytic streptococci. Samples of dust frequently showed tens of thousands around a single bed. Air cultures on a "streptococcus sore throat" ward for 4 consecutive periods—quiet, during sweeping, quiet, during bedmaking—showed striking increases in hemolytic streptococci during sweeping and bedmaking. Daily air cultures in several locations in the ward showed that fluctuations in the number of hemolytic streptococci in the air closely approximated those of the total saprophytic (*i.e.*, floor and bedclothing) bacteria.

Streptococci expelled by the patient or carrier usually fall rapidly to the floor or bedclothing, which become important secondary reservoirs of these microorganisms. Cultures of saliva of persons with "positive" throat cultures often show hundreds to millions of hemolytic streptococci per milliliter, especially in the acute stages of infection. Nasal cultures frequently show large numbers. Moreover, the actual number of hemolytic streptococci recovered from bedclothing of patients with "positive" nose cultures was approximately one hundred times as great as from those in whom only the throat or saliva were positive.

The numbers of hospital cross infections caused by different types of hemolytic streptococci paralleled the numbers of each type recovered from the air.

* From the Commission on Air-Borne Infections, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the United States Army, Preventive Medicine Service, Office of the Surgeon General, U. S. Army.

Hyaluronidase Concentration and Spermatozoa Concentration in Semen as a Gauge in Fertility. By NICHOLAS T. WERTHESSEN, SAUL BERMAN, and BORIS E. GREENBERG (introduced by J. E. F. Riseman), Boston, Mass.

The demonstration of the presence of hyaluronidase in the seminal fluid and its probable function in fertilization (dissolution of the egg's corona) has provided another factor with which to estimate the fertilizing capacity of a particular semen specimen.

A technic, based on the reduction of viscosity of hyaluronic acid by hyaluronidase, has been developed. Accuracy of the method was considered as ± 7 per cent when the enzyme concentration was expressed as an arbitrary unit with 0.1 ml. of semen as the reference volume.

The concentration of the enzyme has been found to be related to the sperm concentration in linear fashion. The equation $M=0.493 + 0.858H$ where M=Millions of sperm per ml. and H the concentration of enzyme (both as logarithmic units) was found to be the best expression of the relationship.

Since it was found that, even with elaborate precautions in counting, the error of a semen count for a single specimen had to be considered as 20 per cent, it is suggested that the use of the hyaluronidase index would provide a better gauge of therapies designed to raise the rate of spermatozoa production than counts of the sperm population.

Work Capacity and Blood Sugar in Adrenocortical Deficiency: Effect of Pork Adrenal Cortex Extract. By CYRIL M. MACBRYDE and (by invitation) F. A. DE LA BALZE, St. Louis, Mo.

Studies were done upon 6 patients with Addison's disease who had severe hypoglycemia and muscular weakness despite adequate control of salt metabolism and blood pressure with desoxycorticosterone or beef adrenal cortex extract. Pork cortical extract, when compared in parallel experiments with beef cortical extract, gave the following results:

(*1*) Better absorption of ingested glucose.

(*2*) Prevention of the hypoglycemic phase at the second or third hour in oral or intravenous glucose tolerance tests.

(*3*) Prevention of blood sugar fall during prolonged fasting.

(4) Intramuscular injection of pork extract produced a fall in the venous blood sugar, reaching a maximum in 2 or 3 hours. This was puzzling until we found that it was accompanied by a rise in the capillary (arterial) blood sugar. No such changes occurred with comparable doses of beef extract. The greater arterio-venous difference in the blood sugar following pork extract apparently reflects an increased utilization of glucose by the muscles.

An increase in glucose metabolized by the muscles should result in greater work capacity. Two types of work experiments were performed to test this hypothesis: finger ergometer, and stair-climbing. Performance was improved in all trials with pork extract, the increase averaging 40 per cent. With beef extract, there was no significant improvement. The extreme fatigue following work in the control tests and after beef extract was not present after pork extract.

Capillary and venous blood sugar determinations before and during the work experiments indicate that the pork extract caused release of liver glycogen and promoted muscle utilization of glucose. The greater effect of the pork extract is apparently due to its higher content of steroids with O or OH on C_{11}.

The Hemodynamics of Neurogenic Hypertension. By RICHARD J. BING (introduced by E. Cowles Andrus), Baltimore, Md.

The hemodynamics of neurogenic hypertension was investigated in a series of 6 unanesthetized dogs. Cardiac output was measured after the method of Fick by inserting a catheter into the right ventricle; mean blood pressure was determined with the Hamilton manometer. P-amino-hippuric acid and creatine clearances were used to obtain renal plasma flows and filtration rates respectively. Changes in the blood flow through the fore-limb were followed with the plethysmograph. The total peripheral resistance, renal resistance, and limb resistance were derived with the formula of Aperia.

After having established control values, the animals were made hypertensive by the Heymans operation. Changes in the cardiac output, renal plasma flow and filtration rate, and in the blood flow through the limb were followed at regular intervals.

The most striking change accompanying the development of neurogenic hypertension was an increase in the heart rate and the cardiac output, the systolic discharge remaining constant. The renal blood flow and filtration rate showed no significant change, while the flow through the limb rose in every instance. The total peripheral resistance rose slightly in 2 animals and remained constant in the rest. The renal resistance, on the other hand, increased more than 50 per cent while the vascular resistance in the forelimb fell.

These results indicate that the development of neurogenic hypertension is accompanied by a rise in cardiac output, constriction of the renal afferent arterioles, and accelerated circulation in the extremities. They are indicative of increased sympathetic tone.

*Laryngeal Epilepsy Due to Increased Intrathoracic Pressure.** By ROBERT W. WILKINS and (by invitation) CARL K. FRIEDLAND, Boston, Mass.

Certain patients with pulmonary disease after severe paroxysms of coughing or laughing may temporarily lose vision and even consciousness. Some normal subjects studied during the Valsalva experiment (inhaling deeply and then forcibly expiring against a closed glottis) also temporarily lost vision. In such subjects, arterial pressure (measured by the Hamilton method) varied as follows: with the onset of the strain it rose sharply (presumably due to the increased intrathoracic pressure). After 2 or 3 seconds, arterial and especially pulse pressure began to fall, and with the "blackout" reached low levels. In others, while the strain was still held, there was a recovery of the arterial pressure toward higher levels. On releasing the strain, there was a brief sharp drop in arterial pressure (presumably due to the removal of intrathoracic support) followed by rapid rise to hypertensive levels. The pulse rate usually varied inversely as the arterial pressure. In certain subjects who "blacked out," the cardiac output judged by ballistocardiograms and roentgenograms decreased markedly during the test.

Two patients with pulmonary disease who complained of "blackout" after coughing showed similar results during the Valsalva test. In one of them, the effect could be simulated by voluntary coughing. These results indicate that this form of "laryngeal epilepsy" is due to preventing venous return to the heart by increasing intrathoracic pressure, with the result that cardiac ouput is so reduced that an adequate arterial pressure cannot be sustained.

*The Effect of Increased Intra-Abdominal Pressure Upon Renal Function in Normal Human Subjects.** By STANLEY E. BRADLEY *and* GERALDINE P. BRADLEY (introduced by C. S. Keefer), Boston, Mass.

Renal function was studied by clearance and saturation methods (Smith) before, during, and following the sudden elevation of intra-abdominal pressure produced by inflating a snugly fitting pneumatic corset to 80 mm. Hg. Intra-abdominal pressure measured under similar conditions by means of duodenal or rectal balloons and by direct catheterization of the inferior vena cava or renal vein revealed increments of 15 to 30 mm. Hg when the abdominal wall was as relaxed as possible. Voluntary bearing-down resulted in a further increase in the pressure, as high as 110 mm. Hg in a muscular individual.

Following the rise in intra-abdominal pressure, the urine flow decreased sharply, the concentration of the urinary solutes (inulin, mannitol, diodrast, and sodium p-amino hippurate) increased, and a moderate proteinuria was noted on several occasions. The rate of urine flow

* This work was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Massachusetts Memorial Hospitals.

tended to return slowly to the control level during prolonged application of the pressure belt.

The glomerular filtration rate and the effective renal plasma flow fell immediately, at the same time and to the same extent, so that the fraction of plasma filtered at the glomerulus remained unchanged through the period of increased pressure. Here, again, restitutive processes were apparent in the gradual return of the filtration rate and effective renal plasma flow toward the base-line. Following release of the pressure, all values returned to the control levels. Reactive hyperemia was not observed.

It was also found that the maximal rates of tubular reabsorption of glucose and excretion of diodrast were reduced during the period of elevated intra-abdominal pressure to the same extent as the plasma flow, so that the ratio between renal blood flow and the mass of functioning tubular tissue as measured by glucose and diodrast Tm remained unaltered. This change in maximal tubular activity rates could not be ascribed to loading reduction secondary to the fall in blood flow and filtration rate since the calculated load/Tm ratio was maintained above 1.2 in all instances. Again, a gradual shift toward the base-line level was observed during long periods of increased pressure and a rapid return to control values followed release of the pressure.

Thus, it appears that increasing the intra-abdominal pressure results in the total cessation of urine formation in a large segment of renal tissue.

*The Capillary Circulation of the Normal Dog.** By John G. Gibson, 2nd, and (by invitation) Arnold M. Seligman, and Wendell C. Peacock, Boston, Mass.

The capillary content of plasma was determined by means of radioactive-iodo-protein, and of red cells, by radioactive iron and hemoglobin measurements on 5 normal dogs under morphine narcosis. Plasma volume was measured by the dye method and red cell volume by radioactive iron.

Only one-fifth of the total blood is in circulation through the capillary bed at one time and not over 5 per cent of total blood is in the capillaries of any organ. The relative ratio of capillary blood content of whole organs is liver and muscle, 6; spleen and gut, 3; lung, kidney, and skin, 2; and, heart and brain, 1.

The hematocrit of capillary blood in the spleen is always greater and that of all other organs is less than the hematocrit of arterial blood. Values for liver, lung, muscle, and skin range from 32 to 38; for kidney, heart, and brain, from 20 to 29; and for bowel, 12. The average arterial hematocrit was 45 and the average body hematocrit was 40.

All the red cells in the capillary bed are in motion at all times in the resting state. No evidence for the existence of any considerable "blood depots" (excluding mesentery and bone marrow which were not measured) was found.

* Work done under contract with the Office of Scientific Research and Development.

The Treatment of Hyperthyroidism with Thiouracil. By Louis J. Soffer and (by invitation) Morley Kert and Lester Gabrilove, New York, N. Y.

Twenty-five patients with well-defined Graves' disease were treated with thiouracil for a period varying from 6 weeks to 6 months. For the first 4 weeks, the dosage was 1 gram daily, divided into 5 doses, and 2/10 gram daily thereafter. Weight, pulse, blood pressure, basal metabolic rate, circulation time, electrocardiographic tracing, magnesium partition, complete blood counts, liver function studies, and renal function studies were recorded before and during the course of thiouracil therapy. The patients were carefully observed for evidence of sensitivity to the drug.

Five patients showed signs of drug toxicity, 2 developed a mild leukopenia, 1 a rash and fever, 1 edema, and 1 conjunctivitis. Approximately ⅔ responded well to thiouracil therapy, with lowered basal metabolism and pulse rates, an increase in weight, and subsidence of the symptoms of hyperthyroidism, although the tremor and eye signs showed no decrease. Nine subsequently came to operation (thyroidectomy). As far as could be determined microscopically, the extirpated glands in the patients who received combined thiouracil and Lugol therapy appeared identical with those of patients who received Lugol's solution alone. There was no evidence of increased hepatic, renal, or cardiac injury as a result of the treatment.

Thiouracil in the Treatment of Thyrotoxicosis. By Robert H. Williams and (by invitation) Howard M. Clute, Boston, Mass.

One hundred and five patients with thyrotoxicosis have been treated with thiouracil for from 5 days to 1 year. One third have been treated for more than 6 months. Within 4 to 5 weeks, there was a clinical remission of the disease with a drop in the basal metabolic rate to normal. Patients who were given iodine before thiouracil treatment responded more slowly. Sometimes thiouracil caused a transitory exacerbation of exophthalmos in patients with malignant exophthalmos. Usually the size of the thyroid gland became definitely smaller after one or more months of thiouracil. In a few cases, estimations of the protein bound iodine of the plasma revealed a normal concentration after a few weeks of treatment. The plasma iodine became normal before the metabolic rate did.

The complications of thiouracil therapy have consisted of agranulocytosis (1 case), morbilliform rash, urticaria, fever, arthritis, swelling of the legs, and enlargement of the submaxillary salivary glands. These complications have been quite rare with reduction in dosage of thiouracil.

Although a satisfactory response in the thyrotoxic state occurred, 30 patients were subjected to thyroidectomy. The operative and post-operative course was relatively smooth, particularly in the patients treated with thiouracil for 3 weeks or longer preceding the operation. A chemical analysis of the thyroid glands

removed showed a great variation in the concentration of thiouracil, but there was no correlation with the therapeutic response. The content of thyroxin iodine in the gland was extremely low, being almost absent in a few cases. Histological changes were quite unlike those of iodized thyroid glands. There was marked cellular hyperplasia, disappearance of colloid, increased interstitial tissue, and scattered infiltration with lymphocytic germinal centers.

The most desirable dosage of thouracil has not yet been established. However, we have found that this drug is very rapidly absorbed from the gastro-intestinal tract and is disseminated throughout all of the tissues and fluids of the body. More than one-half of the ingested drug is broken down in either the gastro-intestinal tract or the tissues of the body, the remainder being excreted at a fairly rapid rate in the urine. Patients can be satisfactorily treated with thiouracil without performing estimations of its concentration in the blood.

*The Effect of Thiouracil on Hyperthyroidism and on the Uptake of Radioactive Iodine in Patients with Adenocarcinoma of the Thyroid and Functioning Metastases.** By Louis Leiter and (by invitation) S. M. Seidlin, L. D. Marinelli, and E. J. Baumann, New York, N. Y.

The action of thiouracil was studied in 2 patients with adenocarcinoma of the thyroid, extensive pulmonary and osseous metastases, and moderate to severe hyperthyroidism. In one case, there was no significant thyroid tissue in the neck; in the other, the non-cancerous right lobe was excluded as a possible source of hyperthyroidism. The former responded to large doses of iodine with a partial remission of the signs of hyperthyroidism; the latter was, if anything, rendered more hyperthyroid by iodinization.

Thiouracil produced a typical remission in both patients, as evidenced by a striking fall in basal metabolism, gain in weight, increase in serum cholesterol, and decrease in organic blood iodine. No untoward reactions were observed. The beneficial effect lasted only during the period of administration of the drug.

The uptake of radioactive iodine by the metastatic thyroid nodules was used as a criterion of functional activity. The effect of thiouracil on this process will be described.

Observations on the Administration of Vitamin P in Cases of Rheumatic Fever. By J. F. Rinehart and (by invitation) Helen Johnson, San Francisco, Calif.

Eleven children with active acute rheumatic fever have been treated with crude hesperidin or related flavone containing substances. Seven children received crude hesperidin orally in doses of 0.75 to 1.5 grams daily and 4 received other substances of supposed vitamin P activity. Four of the cases had shown persistent activity of the rheumatic process for periods of 2 to 4 months at the time the treatment was instituted. All cases exhibited improvement with progressive slowing of the sedimentation rate following the therapy. Significant slowing of the sedimentation rate occurred in most instances within 2 weeks. Graphic representation of the sedimentation rates strongly suggests that improvement was related to this therapy, although it is evident that a larger series of cases must be studied. Observations on capillary permeability are briefly reported.

The use of vitamin P in rheumatic fever is not without a rational basis. Experimental studies have suggested that vitamin C deficiency may be a factor in the pathogenesis of rheumatic fever. Vitamin P was postulated by Szent—Györgyi as a substance which acted in conjunction with vitamin C in maintaining a normal state of permeability in the capillary wall. While dietary experiments in animals have not shown conclusively that vitamin P is an essential food factor, a number of clinical observations have supported its physiological activity in certain types of purpura. Purpuric manifestations are not uncommon in rheumatic fever. In view of the data suggesting the operation of an allergic influence in rheumatic fever, it is noteworthy that the reputed therapeutic value of vitamin P has been in cases of "vascular" purpura including types which are believed to be allergic.

Etiological and Clinical Studies on Endemic Exudative Tonsillitis and Pharyngitis. By Commission on Acute Respiratory Diseases, Station Hospital, Section 2. (Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon-General, U. S. Army). Presented by Elias Strauss (introduced by Charles H. Rammelkamp), Fort Bragg, N. C.

The B-hemolytic streptococcus is generally considered to be the commonest cause of exudative tonsillitis and pharyngitis. In such cases, the presence of the organism is thought to be associated with a characteristic clinical pattern. The present study casts doubt on the validity of these concepts. A group of 116 men admitted to a military hospital because of exudative pharyngitis or tonsillitis were studied. Only 56 of the patients (48 per cent) had B-hemolytic streptococci in one or more throat cultures. Furthermore, only 28 of the 56 developed a significant increase in streptococcal antibodies (antistreptolysins) during convalescence. These 28 patients, constituting 25 per cent of the whole, also differed from the rest in the numbers of streptococci present in cultures, in the amount of exudate in the throat, in the height of the leukocyte response, and in the clinical pattern of the illness. In contrast, patients with streptococci but without an antibody response resembled cases of exudative pharyngitis without streptococci. Both of the latter groups bore a clinical resemblance to cases of undifferentiated respiratory disease.

* Aided by a grant from the Dazian Foundation for Medical Research.

Twelve per cent of all patients had roentgen and laboratory evidence of primary atypical pneumonia. These cases were distributed throughout the whole group. Exudative pharyngitis, whether due to B-hemolytic streptococcus or not, may be associated with or superimposed on other forms of respiratory disease.

*Reversible Inactivation of the Substance Inducing Transformation of Pneumococcal Types.** By MACLYN MCCARTY (introduced by O. T. Avery), New York, N. Y.

Recent studies on the chemical nature of the substance inducing transformation of pneumococcal types have provided strong evidence that this substance is a nucleic acid of the desoxyribose type. Purified preparations of desoxyribonucleic acid isolated from Type III pneomococci are capable in minute amounts of causing unencapsulated variants of Pneumococcus Type II to acquire the capsular structure and type specificity of Pneumococcus Type III. These facts suggest that nucleic acids in general may possess biological specificity. In an attempt to obtain information concerning the chemical basis of this specificity, the reversible inactivation of the transforming substance has been studied.

Ascorbic acid causes rapid and complete inactivation of the transforming substance. Among other compounds tested, only those having the di-enol configuration of ascorbic acid have proved effective in inactivation, and in each case traces of copper catalyze the reaction.

In the presence of glutathione and certain other sulfhydryl compounds, ascorbic acid inactivation is prevented. Moreover, transforming material which has been completely inactivated by ascorbic acid can be quantitatively reactivated by treatment with glutathione. Thus, the specific substance presumably possesses chemical groups which are susceptible to reversible oxidation and reduction and which are present in the reduced state in the biologically active molecule.

Filtering Action of the Guinea Pig Spleen for Foa-Kurloff Cells. By ROBERT W. HEINLE and (by invitation) DAVID K. HEYDINGER, Cleveland, Ohio.

Increase of Foa-Kurloff cells in the blood of guinea pigs after estrogen administration and during pregnancy is a recognized phenomenon. Normal and splenectomized guinea pigs were given diethylstilbestrol in daily subcutaneous doses of 0.01 and 0.05 mgm. Spleens of such animals contained large numbers of Foa-Kurloff cells. Both groups of animals showed a peak of Foa-Kurloff cells in the blood with return to normal numbers in spite of continuous injection. Splenectomized animals reached the peak sooner, the peak was higher, and the duration of the response was longer than in guinea pigs with spleens.

The Foa-Kurloff cell has been described variously as a monocyte or lymphocyte. Results of this experiment indicate it is neither but is probably a reticulo-endothelial cell produced by the stimulus of estrogen. The spleen apparently removes considerable numbers of these cells from the blood stream. Lymph nodes and other reticulo-endothelial organs did not exhibit this filtering mechanism, even in splenectomized animals.

Orthostatic Hypotension in Normal Young Men Following Physical Exertion, Environmental Thermal Loads, or Both. By LUDWIG W. EICHNA and WILLIAM BENNETT BEAN, Fort Knox, Ky.

Symptomatic, and usually disabling, orthostatic hypotension was a common occurrence in healthy young men subjected to strenuous physical exertion or hot environments or both. The exertions ranged from severe, quickly exhausting effort to prolonged endurance hikes. The climatic stresses consisted of hot, dry (120° F., relative humidity 15 to 20 per cent) and hot, humid (90° F., relative humidity 95 per cent) environments.

The syndrome occurred with equal frequency in fit and unfit men and its manifestations were similar, regardless of the inducing stress. Bradycardia occurred often and in 2 men progressed to asystole which in one man persisted for 19 seconds. The duration of the hypotension varied from: (*a*) a fraction of an hour to several hours after short severe exertion, (*b*) several hours to half a day after moderate prolonged exertion, (*c*) one to several days during rest and work in hot environments. The hypotension induced by a specific stress disappeared with repetition to the stress when it was exertion or with a continuation of it when thermal (acclimatization). It reappeared when the stress (especially thermal) was repeated after a long lapse. While erect, the symptoms and circulatory manifestations were prevented or, when present, alleviated rapidly by (*a*) moving the legs, (*b*) occluding the arterial circulation to the legs, and (*c*) removal to a cool room when the stress was thermal.

Orthostatic hypotension is a common occurrence in normal men subjected to various stresses and represents one of the manifestations of the failure of the circulation to cope with the load imposed.

Hemolytic Reaction Due to Rh Incompatibility Following First Transfusion in a Woman Eight Years After Birth of an Erythroblastotic Child. By LAWRENCE E. YOUNG, *and* DONALD H. KARIHER (introduced by John S. Lawrence), Rochester, N. Y.

It is now accepted that many transfusion reactions occurring during pregnancy or the post-partum period are due to the development of Rh antibodies in Rh negative women who are carrying or have recently given birth to Rh positive children. However, the danger of transfusing such women with Rh positive blood many years later is not fully realized. A recent case studied in the Strong Memorial Hospital emphasizes this hazard.

A radical mastectomy was performed November 15, 1943, on a 40-year-old woman, blood group A1M Rh

* The Bureau of Medicine and Surgery of the U. S. Navy does not necessarily undertake to endorse views and opinions expressed in this abstract.

negative, who had given birth to an Rh positive erythroblastotic child February 7, 1936. No anti-Rh agglutinins could be demonstrated prior to operation. Postoperatively she received 500 ml. of blood from a donor whose group was A1MN Rh positive. This was followed by a mild chill, oliguria for 3 days, marked azotemia, and complete destruction of the donor's cells within 11 days as demonstrated by MN tests. Her anti-Rh agglutinin titer rose to 1:32,000 on the 24th day after transfusion.

It is recommended that Rh negative women receive only Rh negative blood, regardless of matching tests and regardless of the interval since possible sensitization to the Rh factor.

The Reaction in Humans to Phosphatide Fraction of Human Tubercle Bacilli. By GEORGE T. HARRELL, Winston-Salem, N. C.

The tubercle bacillus is one of the rare micro organisms which produce a similar reaction in the body if injected dead or alive. This is due in a large measure to the high content of lipids which are a distinguishing feature of the organism. The lipids have been little investigated biologically. R. J. Anderson, Yale University, has chemically analyzed the lipid fractions of tubercle bacilli. Florence Sabin has tested the biologic reactivity in guinea pigs and rabbits. In the study of human cases of sarcoid, the resemblance of the lesions in pathologic sections to photomicrographs accompanying Sabin's paper was striking. Lipid fractions were obtained from Anderson, the same materials used by Sabin, and injected intradermally in humans with tuberculosis, sarcoid, and normals. In tuberculars, the reaction to phosphatide was marked, with the production of giant chronic wheals which appeared on the second day and persisted for as long as 5 days. Patients with sarcoid and normal individuals did not exhibit this response. Wheals are usually acute reactions persisting only minutes, and may be produced by protein or carbohydrate materials. Since the phosphatide is a complex lipid containing a carbohydrate radical, it might be possible that the chronic wheal is produced by gradual breakdown in the tissues of the phosphatide and liberation of a carbohydrate fraction. This could act as a haptene. Microscopically, the reaction is characterized by an acute reaction with polymorphonuclear neutrophiles and eosinophiles, epithelioid cells, but no tubercles. In certain individuals, a late reactivation occurred in the second week at the sight of injection. This may represent the development of sensitivity to phosphatide or some breakdown product.

The Treatment of Syphilis in Six to Twelve Weeks by Triweekly Injections of Mapharsen: An Evaluation of the First 4800 Cases. By HARRY EAGLE, Baltimore, Md.

Early syphilis can be treated effectively and with reasonable safety by the administration of mapharsen at a unit dosage of approximately 1 mgm. per kgm., repeated 3 times weekly for a total of 8 to 12 weeks. The end results on this schedule were comparable with those obtained with routine 18 months' treatment, and considerably better than those obtained in average clinic practice. The concurrent administration of bismuth subsalicylate, given intramuscularly once weekly at a dosage of 0.2 gram, had a markedly favorable effect on the final outcome, the estimated "cure" rate in such cases being approximately 90 per cent. Although the best results were obtained in patients receiving a total mapharsen dosage of 27 to 35 mgm. per kgm. supplemented by weekly injections of bismuth, there was but little difference between patients on that dosage and those receiving 21 to 27 mgm. per kgm. mapharsen. Reduction in the total amount of mapharsen below that level caused a significant increase in the proportion of treatment failures.

The results were somewhat better in primary than in secondary syphilis, with no significant difference between seronegative and seropositive primary syphilis. Race, sex, or age had no demonstrable effect on the outcome of treatment.

The Diagnosis of Raynaud's Disease in Men. By E. A. HINES, JR., and (by invitation) N. A. CHRISTENSEN, Rochester, Minn.

It has been assumed that Raynaud's disease occurs rarely in men, for it is usually estimated that from 90 to 97 per cent of the cases are among women. When Raynaud's phenomenon occurs in men, it is almost always considered secondary to some disease, such as thromboangiitis obliterans, which, if it cannot be immediately diagnosed, almost always will become evident when enough time has elapsed. As far as we know, a study of the adequacy of the diagnosis of Raynaud's disease has not been made in a large group of men and the verification of the diagnosis by an adequate follow-up study has not been carried out.

From 1920 to 1942 inclusive, the diagnosis of Raynaud's disease or questionable Raynaud's disease was made in 830 cases at the Mayo Clinic; 649 patients (78 per cent of the total group) were women and 181 (22 per cent) were men. The purpose of our study has been to try to evaluate the correctness of the diagnosis in the group of 181 men and to verify or disprove the diagnosis by a follow-up study carried out 2 or more years (average 8 years) after the onset of the Raynaud's phenomenon.

The criterion for the diagnosis of Raynaud's disease which we used in our study was similar to that suggested by Raynaud and by Hutchinson and was that outlined by Allen and Brown in 1930.

In 111 of the 181 instances of the disease in men, the diagnosis made at the time of the patient's original visit was Raynaud's disease and in 70 cases it was questionable Raynaud's disease. From our analysis of the data, we considered that the diagnosis of Raynaud's disease was adequate in 58 of the 181 cases, that it should have been questionable Raynaud's disease in 93 for such reasons as short duration of symptoms, unilaterality of Raynaud's phenomenon, unrecorded or questionable presence of

arterial pulsation, and the possibility of primary diseases which might be causal and that it was almost certainly incorrect in 30 cases. Thus, in our opinion, 30 patients (17 per cent) received an incorrect diagnosis of Raynaud's disease at the original visit to the clinic and 58 (32 per cent), a correct diagnosis of Raynaud's disease. This is an unexpectedly large number of men for whose condition the diagnosis of Raynaud's disease made at the time of their original visits could be considered correct by accepted criteria. This observation is especially significant inasmuch as the diagnosis usually had been made by clinicians who were not especially experienced in the field of peripheral vascular disease.

We were able to obtain satisfactory follow-up information either by letter or by re-examination concerning 100 of the 181 men. Our study of the original case records of these 100 men led us to conclude that the diagnosis of Raynaud's disease which was made at the time of the original visit was adequate in 34 cases, questionably adequate in 54, and almost certainly incorrect in 12. On analysis of the follow-up data, it was considered that the diagnosis of Raynaud's disease could now be made unquestionably in 59 cases, that the diagnosis was still questionably adequate in 9, and that it was incorrect in 32. Thus in 59 (67 per cent) of the 88 cases in which the original diagnosis should have been Ryanaud's disease or questionable Raynaud's disease, the follow-up study verified a diagnosis of Raynaud's disease, according to the usually accepted criterion for making this diagnosis.

These data indicate that Raynaud's disease occurs more commonly in men than is usually considered and that if the criterion as emphasized by Allen and Brown is followed in making the original diagnosis of Raynaud's disease, the diagnosis, when made in men, will be verified by the passage of time in a high percentage of cases.

*The Influence of Humidity on the Survival of Influenza A Virus in Air and Dust.** By CLAYTON G. LOOSLI, ELIZABETH APPEL, HENRY M. LEMON (by invitation), and O. H. ROBERTSON, Camp Carson, Colo.

Influenza A infections in mice can be readily produced by allowing them to breathe air contaminated with the virus. The virus-laden atmospheres were produced (*1*) by spraying suspensions of virus (PR8 strain) prepared from infected mouse lungs, as fine droplets into a room of 800 cubic feet capacity, and (*2*) by raising dust from the floors of the room into which virus had been sprayed many hours or days previously. With the introduction of approximately the same amount of virus suspension at a given temperature (27° to 30° C.) but at varying relative humidities, it was found that atmospheres with humidities of from 80 to 90 per cent were infective for mice for only 1 hour, while those at 45 to 55 per cent were infective for 6 hours. Atmospheres of low humidities (17 to 30 per cent), however, were infective for as long as 48 hours and the virus could be isolated from the dust 5 days after its introduction into the room air. Lethal concentrations of virus suspended in atmosphere of low humidity and present in the floor dust were rendered noninfective by raising the humidity 65 per cent or higher for 2 hours. Atmospheres containing insufficient virus to produce influenza in mice as a result of a 20-minute exposure were found to be highly infective if the mice were allowed to remain in the virus-containing air for 1 hour. These observations support the accumulating evidence that contaminated air may be an important vehicle for the spread of infectious agents and suggest certain possibilities as to their control.

* From the Department of Medicine, University of Chicago, Commission on Influenza and Commission on Air-Borne Infections, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the U. S. Army, Office of the Surgeon General, Preventive Medicine Service.

Effects of Dilution and of the Addition of Dextrose on the Rate of Hemolysis in Stored Blood. By ELMER L. DEGOWIN and (by invitation) IRVING FRIEDMAN, Iowa City, Iowa.

It has been previously reported that the modified Rous-Turner mixture of 10 volumes of blood, 2 volumes of 3.2 per cent sodium citrate, and 13 volumes of 5.4 per cent dextrose solution permitted storage of blood for transfusion for 30 days at 2 to 5° C. Many other dextrose-citrate mixtures have been introduced in attempts to secure maximum preservation with reduced bulk. This object is particularly desirable when problems of military transportation are encountered.

In blood-dextrose-citrate mixtures with a blood:diluent ratio of 1:0.3 varying the dextrose concentration from 0.38 to 4.9 per cent resulted in no significant difference in the rate of spontaneous hemolysis during storage at 2 to 5° C. for 30 days. Mixtures with blood:diluent ratios from 1:0.3 to 1:2.3 were studied in which the rate of spontaneous hemolysis increased proportionately when the following diluents were employed with blood-citrate: sodium chloride, sodium citrate, human albumin + sodium chloride, human albumin, sucrose, and dextrose. Minimum hemolysis was obtained during 30 days with dextrose and albumin. The addition of dextrose in the concentration of 200 mgm. per cent improved storage in all mixtures. The use of electrolytes increased the rates of hemolysis.

The formation of inorganic phosphorus in the erythrocytes was much greater when dextrose was not added but this fact could not be correlated with the inhibition of hemolysis by sucrose.

It is concluded that the addition of dextrose may serve a dual function in the preservation of blood, by acting as a substrate for glycolysis and by dilution of the plasma with a non-electrolyte. Dilution of the plasma is an important factor in the preservation of blood. The optimum dilution is in excess of a blood:diluent ratio of 1:1.

Simultaneous Arterial and Venous Blood Cultures in Cases of Bacterial Endocarditis. Evidence on the Sites of Removal of Bacteria from the Circulating Blood. By PAUL B. BEESON and (by invitaiton) JAMES V. WARREN and EMMETT S. BRANNON, Atlanta, Ga.

Observations were made on 5 patients with bacterial endocarditis, in an attempt to ascertain the places where bacteria are removed from the circulating blood. Colony counts in cultures of arterial blood were compared with colony counts in venous blood taken at the same time. The venous samples were drawn not only from superficial and deep vessels of the extremities, but also from the superior and inferior vena cava, the right auricle, the hepatic and renal veins. To secure these samples from the venous circulation of the trunk, a flexible ureteral-type catheter with an angled tip was passed, under fluoroscopic guidance, through an arm vein into the superior vena cava and right auricle, whence it could be guided into the other vessels mentioned.

It was found that mixed venous blood in the right auricle usually contained only about half as many bacteria as were present in arterial blood. During periods of 1 or 2 hours, serial specimens of arterial blood showed relatively constant colony counts; thus it appears that in bacterial endocarditis, organisms are being discharged into the circulation more or less steadily. Blood from the hepatic veins always gave strikingly low counts, sometimes only 2 to 5 per cent of the corresponding arterial level. In the superior vena cava, the renal and femoral veins, colony counts were from one-half to three-fourths as high as in the corresponding arterial samples. On the other hand, there was little difference between the colony counts in arterial and antecubital vein blood.

*Physiologic Effects of Purified Gas Gangrene Toxins.** By AUSTIN M. BRUES, and (by invitation) ALFRED POPE, PAUL C. ZAMECNIK, ABBY L. NUTT, IRA T. NATHANSON, and JOSEPH C. AUB, Boston, Mass.

Because of the frequency of clostridia in battle wounds and the readiness with which they multiply and produce toxin in anoxic muscle, we have investigated the possible influence of their toxins in shock. Experiments have been done on dogs, with continuous recording of the blood pressure and oxygen consumption, determinations of hematocrit, plasma protein concentration, cardiac output, peripheral vascular resistance, venous pressure, and plasma volume. Local fluid loss was estimated by measurements of leg volume, and electrocardiograms have been made.

Following intravenous injection of *Cl. perfringens* toxin (above 200 mouse m.l.d.), blood pressure declines sharply and there is massive intravascular hemolysis followed by escape of hemoglobin from the plasma. Smaller doses result in a transitory drop in blood pressure and partial hemolysis.

Intramuscular toxin may also be fatal, but with little or no hemolysis; death can be attributed to shock due to local loss of intravascular fluid. Only 12 mouse m.l.d. injected into one gastrocnemius has caused local loss of approximately 18 per cent of the plasma. This suggests that minimal local gas gangrene infection might produce or aggravate the shock syndrome due to loss of circulating plasma.

* Work done under contract with the Office of Scientific Research and Development.

Penicillin in the Treatment of Meningitis. By DAVID H. ROSENBERG and (by invitation) PHILLIP A. ARLING, Great Lakes, Ill.

The clinical effectiveness and minimum adequate dosage requirements of penicillin were studied in 57 patients with cerebrospinal fever (13 with meningococcemia), in 2 patients with hemolytic streptococcal meningitis (1 with bacteremia), and in 1 patient with streptococcus viridans meningitis with bacteremia.

Penicillin, administered both intrathecally (10,000 units every 24 hours) and parenterally (10,000 to 15,000 units every 3 hours), produced a rapid clinical response with recovery in 59 out of 60 patients. The one fatality occurred in an individual who was moribund on admission and who presented signs of well advanced meningitis with meningococcemia and secondary hydrocephalus.

In the majority of instances, 10,000 to 20,000 units of penicillin intrathecally resulted in recovery, whereas in the more fulminating types of infection 30,000 to 50,000 units were required. Meningococcemia was controlled with 40,000 to 250,000 units, the maximum dose having been used in the fulminating infections. Sterilization of the blood was found in the patients with streptococcic bacteremia after 110,000 units, but penicillin was continued until 300,000 to 400,000 units were administered. Parenteral penicillin was not effective in the prevention and treatment of acute epididymitis and epididymo-orchitis complicating cerebrospinal fever, nor in the treatment of acute meningococcic fibrinous pericarditis or arthritis.

Factors Influencing Acclimatization and Performance in Humid (Tropical) Heat. By WILLIAM BENNETT BEAN, LUDWIG W. EICHNA, and (by invitation) WILLIAM F. ASHE, and NORTON A. NELSON, Fort Knox, Ky.

A study of heart rate, blood pressure, rectal and skin temperatures, changes in blood and urine, subjective sensations, and related phenomena, has been made in 64 men during acclimatization to work (marching at 2.5 miles per hour with a 20 pound pack) in humid heat (90° to 91° F.; relative humidity 95 per cent). The acclimatized man works with a lower heart rate, lower skin and rectal temperature, more stable blood pressure, and less discomfort than when unacclimatized. Strenuous or protracted work is not well tolerated initially. Acclimatization begins with first exposure, is facilitated by a gradual increase in work, and is nearly complete in 3 to 10 days. Early intolerance to heat does not retard

or prevent acclimatization if rest, water, and salt are supplied. Resting in humid heat induces but little acclimatization. It develops most rapidly in subjects entering the hot room in summer and is retained longest in summer. Cross acclimatization between desert and tropical heat exists. Performance in humid heat is impaired by (*1*) lack of acclimatization, (*2*) added clothing, (*3*) lack of rest and sleep, (*4*) water deprivation (thirst is an inadequate guide to requirement), (*5*) lack of physical fitness, (*6*) alcohol, (*7*) long periods of work, and (*8*) slight increases in humidity and temperature.

*Compensatory Peripheral Vascular Adjustments During Spinal Anesthesia.** By CHARLES NEUMANN (by invitation), ALBERT D. FOSTER, JR. (by invitation), EMERY A. ROVENSTINE (by invitation), and ALFRED E. COHN, New York, N. Y.

The plethysmogram of fingers and toes has two principal waves: (*1*) Pulse waves, and (*2*) alpha waves (rhythmic variations in volume occurring 4 to 8 times per minute). Normally the pulse wave-alpha wave patterns of fingers and toes correspond and change similarly in response to physiologic stresses associated with alterations of sympathetic activity.

To demonstrate the influence of the sympathetic nerves, regional anesthesia was induced by the spinal route in 15 normal individuals. The areas (toes) deprived of sympathetic stimuli increased in volume, pulse waves increased 2 to 3 times and alpha waves disappeared (vasodilatation). Concurrently, areas (fingers) still under the influence of active sympathetic innervation showed decrease in volume, decrease in pulse waves to one-third their former size, and decrease in alpha waves (vasoconstriction). Blood pressure and pulse rate remained constant. These observations suggest that vasodilatation in any considerable peripheral vascular bed is accompanied by compensatory vasoconstriction elsewhere.

In 4 subjects under spinal anesthesia, a fall in blood pressure occurred. It was preceded by relaxation of the usual vasoconstriction in the unanesthetized fingers. Administration of ephedrine to these subjects was followed by return of vasoconstriction in the fingers, and then by elevation of blood pressure. Vasodilatation in the toes was unaltered during the depression of blood pressure or its subsequent rise.

The Relative Constancy of Inulin and Diodrast Clearances. By GEORGE F. KOEPF, ROGER S. HUBBARD, and TED LOOMIS (introduced by David K. Miller), Buffalo, N. Y.

A statistical analysis was made of the results of simultaneous inulin and diodrast plasma clearances obtained in 79 separate 15 minute test periods upon 19 subjects. The experiments were carefully carried out to insure satisfactory collection of blood and urine samples. It was found, when the individual clearance periods were studied, that the inulin clearance, taken to be a measure of glomerular filtration, showed greater constancy than did the diodrast clearance, which is regarded as a measure of the plasma flow to active kidney tissues. The coefficients of variation were; for the inulin clearances: 39 ± 2.4, and for the diodrast clearance: 55.2 ± 3.8. The difference between these values has a 98 per cent probability of being statistically significant. The authors believe that the greater constancy of the inulin clearance arose largely from regulation of the glomerular filtration of the individual subjects, for when the results upon each subject were averaged, and these averages analyzed statistically, the coefficients of variation of inulin and diodrast clearances were 31.9 ± 3.8 and 39.9 ± 5.0, respectively.

Hypersplenism. By WILLIAM DAMESHEK, Boston, Mass.

Following splenectomy, various changes develop in the morphologic elements of the blood. The red cells become thinner and show Howell-Jolly bodies; the white cells and platelets become increased. Splenomegaly is often accompanied by anemia, leukopenia, and thrombocytopenia in the presence of a hyperplastic bone-marrow; following splenectomy, sharp increases in the red cells, white cells, and platelets usually take place. These observations suggest (*a*) a relationship between the bone-marrow and the spleen and (*b*) a possibly enhanced relationship ("hypersplenism") in cases of splenomegaly. In cases of idiopathic thrombopenic purpura without splenomegaly, the marrow is crowded with unproductive megakaryocytes which, following splenectomy, show extreme platelet production. In certain cases of granulocytopenia, with or without splenomegaly, the marrow is crowded with leukocytes; following splenectomy, both the marrow and the blood become normal. These cases, and certain types of hemolytic anemia reacting quickly to splenectomy, suggest the possibility of abnormal types of hypersplenism. Although there is much indirect evidence indicating the presence of splenic hormones, direct evidence is scant. Studies of parabiotic material, the further preparation of splenic extracts from normal and abnormal cases, and the development of anti-splenic serum show promising results in this direction.

Gastric Excretion of Sulfadiazine In Man. Observations on Normals, Patients with Peptic Ulcers, Atrophic Gastritis, and Gastric Cancer. By LEON SCHIFF, NATHAN SHAPIRO (by invitation), and HENRY S. BLOCH (by invitation), Cincinnati, Ohio.

The concentration of sulfadiazine in the blood and gastric juice was determined in 43 subjects following intravenous injection of 5 grams of sodium sulfadiazine in 300 ml. of physiological saline. The gastric juice was obtained by continuous aspiration for a period of an hour and a half or more. The subjects included 7 normals, 15 with peptic ulcer, 8 with atrophic gastritis, and 13 with gastric cancer.

* The Bureau of Medicine and Surgery does not necessarily undertake to endorse views or opinions which are expressed in this paper.

The concentration of sulfadiazine in the gastric juice of patients with gastric cancer and achlorhydria tends to be higher than in normal individuals, patients with gastric or duodenal ulcer, or patients with atrophic gastritis, with or without achlorhydria. The concentration of sulfadiazine in the gastric juice of patients with gastric cancer is roughly proportional to the extent of the tumor.

The Mechanism of Shock Produced by the Injection of Trypsin, Thrombin, and a Proteolytic Plasma Preparation. By HENRY J. TAGNON (introduced by George R. Minot), Boston, Mass.

Crystalline trypsin, *in vitro*, accelerates the coagulation of normal and hemophilic blood. *In vivo*, however, as shown by Dragstedt and Rocha E. Silva, in dogs and rabbits, it prolongs the clotting time and produces shock. The prolongation of the clotting time has been ascribed by these authors to the presence of heparin. The work here presented is an attempt to elucidate by what mechanism the clotting action of trypsin observed *in vitro* can prolong the clotting time *in vivo*, and what part it has in the production of shock.

The data show that the prolongation of the clotting time following intravenous injection of trypsin into dogs and rabbits is accompanied by a marked hypothrombinemia and fibrinogenopenia and is not due to the production of some anticlotting agent.

Thrombin and a homologous plasma preparation having properties, similar to trypsin, which accelerate the clotting time *in vitro*, likewise produce hypothrombinemia and fibrinogenopenia with prolongation of the clotting time when injected intravenously. The mechanism seems to be the promotion of a progressive intravascular coagulation resulting in the consumption of fibrinogen and prothrombin, although no clot may be seen at autopsy. The absence of clot is understandable if the fibrin formed is uniformly deposited on the enormous surface of the endothelial wall. This is further indicated by the preventive action of heparin on the prothrombin and fibrinogen changes following intravenous injection of any of the three clotting agents.

Thrombin differs, however, from the two other agents in that its vaso-depressive (shocking) action is entirely abolished by the preliminary administration of heparin, which renders milder but does not abolish the vaso-depressive action of trypsin and the plasma enzyme preparation. This indicates that the vaso-depressive action of thrombin is abolished when clotting is prevented and is presumably entirely due to its clotting action, which accounts for only part of the vaso-depressive action of trypsin and the plasma enzyme.

Human Requirements for Thiamin and Riboflavin. By VICTOR A. NAJJAR (by invitation) and L. EMMETT HOLT JR., Baltimore, Md.

Experiments have been undertaken on human subjects on synthetic diets in which all B vitamins have been supplied as pure compounds, thus eliminating the error caused by the highly variable quantities of these vitamins in natural foods.

Data have been obtained showing: (*1*) the daily quantities of thiamin and riboflavin required to protect sedentary adults from deficiency, (*2*) the effect of biosynthesis of vitamins by intestinal bacteria on the daily requirement, and (*3*) the effect of varying the composition of the diet on biosynthesis of these vitamins.

Changes Produced by Gonadotropins in Long-Standing Cases of Hypopituitarism. By JACOB LERMAN and (by invitation) IRA T. NATHANSON, Boston, Mass.

The effect of gonadotropins was evaluated in a study of the therapy suitable for patients with hypopituitarism. Two males, ages 46 and 48, showed definite clinical and laboratory response after the administration of chorionic gonadotropin. The clinical change consisted of increased physical energy, change in the psyche, development of libido, erections, and sexual potency for the first time in many years, and enlargement of the genitals and gonads. In one of these patients, administration of thyroid hormone may have enhanced the gonadotropic effect. The laboratory evidence consisted of a definite rise in the excretion in the urine of the 17-ketosteroids during the period of treatment. Testicular biopsies demonstrated slight but definite increase in activity in the tubules.

On omission of therapy, there was a fall in the excretion of the 17-ketosteroids and a regression of the clinical improvement.

One woman, age 47, with profound hypopituitarism (Sheehan's Disease) of 17 years' duration, failed to respond to gonadotropic therapy. This is in contrast to the excellent response from gonadotropic hormone in another patient, previously reported, who was 29 years of age, with the disease of 7 years' duration.

It is concluded that the gonads of adult patients with hypopituitarism respond in some instances to gonadotropic therapy.

Leg Venograms in the Course of Operation for Hysterectomy. By DAVID DAVIS and (by invitation) A. STONE FREEDBERG, Boston, Mass.

In a previous study lower leg venous pressure increases were demonstrated during operations for hysterectomy. To examine the possibility that these increases were due to local spasm of the veins of the legs, 13 venograms were made during 4 operations for hysterectomy using the technic of Bauer.

In one, the venograms showed normal filling of the deep veins. In another, filling was normal except for one small segment. A second venogram taken later showed excellent filling.

In 2 other operations, there were marked filling defects of the deep veins. In one, 5 venograms during and shortly after operation for the removal of a large fibroid showed a changing picture, varying from absent to partial filling with narrowing and breaks in continuity. In the

remaining case, 3 venograms during the operation likewise showed little or no filling of the deep veins, although the saphenous and communicating branches were visualized. Several hours later, a venogram showed good filling of the upper half of the deep veins.

The filling defects noted differed in no way from those obtained in the presence of deep vein thrombophlebitis. They were not considered artefacts, for experience with the Bauer technique has shown that the deep veins fill regularly when the small saphenous vein is properly injected and visualized.

The Effect of Desoxycorticosterone Acetate On the Blood Pressure of Man. By GEORGE A. PERERA, ABBIE I. KNOWLTON, ALICE LOWELL (by invitation), and ROBERT F. LOEB, New York, N. Y.

Attention has been called by many authors to the production of hypertension in animals and in patients with Addison's disease following the administration of desoxycorticosterone esters. It has not become apparent whether the increase in blood pressure is due to salt and water retention or to some other unexplained activity of the hormone.

The effects of desoxycorticosterone acetate (DCA) and of sodium chloride, upon the blood pressure, blood volume, and sodium concentration of the blood, were therefore studied in comparable groups of patients with Addison's disease and in 3 patients without demonstrable adrenal or cardiovascular disease.

No hypertension appeared in 15 patients with hypoadrenalism maintained in electrolyte balance with sodium chloride alone for an average of more than 3 years. Eleven of 23 patients with Addison's disease, maintained in electrolyte balance with DCA, developed blood pressure levels repeatedly in excess of 140/90, 4 of these being excluded from consideration because of the possibility of antecedent cardiovascular disease. The 3 patients with no adrenal disease showed a significant rise in blood pressure following DCA after preliminary control observations.

The elevation of blood pressure could not be correlated with excessive retention of salt water in the circulating blood or to a labile vascular system as manifested by a positive cold pressor test. It therefore seems probable that DCA acts directly or indirectly upon the peripheral vascular system.

A Simple, Rapid, Quantitative Method for Determination of Penicillin. By EDWIN E. OSGOOD and (by invitation) BARBARA GAMBLE, Portland. Oregon.

The method to be described is satisfactory for determination of penicillin in pure solution, penicillin cultures, cerebrospinal fluid, blood serum, urine, or tissue fluids. Results are obtainable within 2 to 3 hours, and it is possible to measure as little as 0.001 unit per ml. The optimal sensitivity is in the region of 0.04 unit per ml. to which all higher concentrations may be diluted. In concentrations between 0.02 and 0.08 units per ml., the accuracy with research care is about plus or minus 5 per cent, and with ordinary clinical technic it is about plus or minus 10 per cent. The method is also suitable for the quantitative comparison of chemotherapeutic agents. The mathematical formulae on which the determination is based give quantitative data on the interrelationship between bacterial numbers and penicillin activity.

Changes in the Electroencephalogram Produced by Sodium Amytal and Sodium Pentothal. By MARY A. B. BRAZIER (by invitation) and JACOB E. FINESINGER, Boston, Mass.

The use of sodium amytal and pentothal in the treatment of war neuroses has stimulated further interest in the mechanism of their action.

Sodium amytal and pentothal when administered intravenously in a series of 20 psychoneurotic patients produced an almost immediate and very marked change in the electroencephalogram which is obvious on gross inspection. It consisted most markedly of the development of high-voltage fast activity (i.e., potentials of 24 to 28 cycles per second and of 150 to 200 microvolts). There was a specificity for waves of this frequency. At higher concentrations of the drug, slow waves of delta frequencies appeared.

The use of a method entailing an accurate measurement of electroencephalograms has been applied to establish the distribution of individual frequencies in these drug records. This method has revealed that the characteristic high-voltage activity appears first and most prominently in the frontal leads, and proceeds gradually to the parietal and occipital leads. On recovery from the drug, the progression is in the reverse direction, and the effect lingers most persistently in the frontals. These waves disappear with the onset of sleep and reappear if the patient is roused. The progression in the cortex is too slow to be explained by differences in cerebral circulation. These waves occur first and are most prominent and persistent in those parts of the cortex which are of the most recent phylogenetic development.

The Leukocyte Count in Primary Atypical (Virus) Pneumonia. By OVID O. MEYER and (by invitation) ETHEL W. THEWLIS, Madison, Wis.

Since the diagnosis of primary atypical pneumonia (virus pneumonia) is necessarily dependent on the clinical and roentgenological findings and the exclusion of other types of pneumonia, rather than by conclusive bacteriological methods, any additional diagnostic aid might be welcome. With the hope of discovering further help in the laboratory, careful detailed studies of the blood counts were made. The usual methods were employed. Initial complete blood counts and hematocrit determinations were made, and thereafter leukocyte counts with differential counts of 500 cells on Kingsley stained smears were done thrice weekly, or oftener, in 50 patients. The characteristic but not invariable findings in the

initial leukocyte counts were as follows: normal total leukocyte count with an increase in neutrophiles and monocytes (large mononuclears) and a lymphopenia; eosinophilia in 26 per cent of the cases that had initial counts made relatively late in the course of the disease.

As the patients improved, the total leukocyte count usually increased, often to above normal level, and the neutrophiles, lymphocytes, and monocytes tended to resume normal relationships.

It appeared that the monocytosis in primary atypical pneumonia was perhaps the most significant of the findings and in association with the other blood findings, might have some diagnostic significance.

Evidence Bearing on the Mechanism of Certain Atrial Arrhythmias. By George Decherd and (by invitation) Arthur Ruskin, Galveston, Tex.

Barker *et al.* have recently summarized the evidence which supports the view that paroxysmal atrial tachycardia is the result of a circus movement. The rather frequent close association of tachycardia with flutter or fibrillation inclined us to the idea that all three arrhythmias might have a similar physiologic basis. This possibility has been put to one test by using a slight modification of the method by which Lewis, Drury, and Iliescu in 1921 demonstrated a circus movement in clinical flutter and fibrillation.

Simultaneous electrocardiographic tracings have been made of two leads in each of three planes in the chest, *i.e.*, frontal, horizontal, and sagittal. These tracings have been enlarged by projection, and from them we have calculated the electrical axes of the atrium for each 1/100-second. These axes have been diagrammatically arranged to show the serial change in the directions of consecutive axes in each plane, and from them three-dimension models have been constructed.

With this procedure, we have studied the curves of the momentary atrial axes in patients with sinus rhythm, with and without various types of heart disease; and in patients with atrial flutter, fibrillation, and paroxysmal tachycardia. The curves derived from flutter show characteristically a return to their initial direction, and form a roughly circular diagram; those from fibrillation show this same essential feature, though they are much more irregular. The curves from paroxysmal tachycardia resemble those from sinus rhythm, differing only in their direction; neither show any tendency to return to their initial direction, or to inscribe a circus. We have interpreted these findings as favoring the view that paroxysmal tachycardia is the result of an ectopic pacemaker, rather than of a circus movement.

The Increase of Cytochrome C Content of Organs Following its Parenteral Injection. By S. Proger and (by invitation) D. Decaneas and G. Schmidt, Boston, Mass.

The influence of the concentration of cytochrome C on the activity of cytochrome oxidase has recently been studied by Stotz, Altschul, and Hogness (1938) and by Schneider and Potter and others (1943). When the results of these investigations are compared with the amounts of cytochrome C and cytochrome oxidase in the tissues, especially in heart, brain, liver, and kidney, it appears that the cytochrome contents in these organs range far below the concentrations required for the maximal activity of the cytochrome oxidase present. It is, of course, possible that the effectiveness of the total cell respiration is determined by limiting factors other than the activity of the cytochrome-cytochromoxidase system. Nevertheless, the apparent existence of suboptimal conditions for the action of this system in normal tissues seems to justify the attempt to investigate the physiological effects of experimental conditions designed to increase the rate of action of cytochrome oxidase as by supplying additional cytochrome.

Cytochrome C, extracted from beef hearts, was injected into rats intravenously, intraperitoneally, and intramuscularly. This results in a definite increase in the content of cytochrome C in the brains, kidneys, livers, and hearts, the pattern of increase and subsequent decrease in the various organs varying, depending upon the mode of administration and experimental conditions such as anesthesia and anoxia.

Cytochrome C, given intravenously to dogs, appears to increase the arterio-venous oxygen difference under conditions of anoxia presumably because it stimulates increased tissue respiration and hence removal of oxygen from the blood stream.

It is suggested that respiratory enzymes such as cytochrome C might be studied further with the hope of employing them in the attack on the problems of tissue anoxia, both acute and chronic.

Acute Respiratory Disease in the Canadian Army. By W. R. Feasby (introduced by W. Hurst Brown), Ottawa, Ontario, Canada.

These studies were conducted, during 1943, at Camp Borden, Ontario, an area where good sampling was possible.

(1) *Losses* for acute respiratory disease in the Canadian Army are calculated for 200,000 men serving in Canada as follows:
- (*a*) Half a million hospital days
- (*b*) One million duty days
- (*c*) $5,800,000 hospitalization costs
- (*d*) Three per cent (of all who fell ill) discharged, and 0.1 per cent dead in 6 months.

(2) *Causes*
- *A. Environmental*—High respiratory sick rates coincided with:—
  - (*a*) Bad weather
  - (*b*) Bad quartering and poor dust control
- *B. Etiological agents*
  - (*a*) *Virus*—In February non-influenzal infections occurred. Twelve per cent of patients had pneumonia. Mice and ferrets had minimal lung lesions. In April, a

small influenza A epidemic occurred. A widespread A epidemic occurred in November.

(*b*) *Streptococcal*—From cases of scarlet fever, tonsillitis, pharyngitis, etc., 1200 streptococcal groupings and typings were done. Up to 85 per cent of acute respiratory infections were associated with group A haemolytic streptococcal infections. Type 19 (17) predominated. Scarlet fever had less type scattering than other streptococcal illnesses. Specific types were not incriminated in the complications, but five out of seven patients with nephritis had type 14 associated. Hospital cross-infections were frequent. Scarlet fever did not produce complications more often than other streptococcal infections. Half of the 100 cases of polyarthritis are now discharged. Two are dead.

(*3*) *Control Measures*

These include dustless sweeping and cleaning of barracks, and proper blanket care, which have been enforced by an educational campaign since June, 1943.

Influence of Aspirin on Urate Excretion. By FRIEDRICH KLEMPERER (by invitation) and WALTER BAUER, Boston, Mass.

Acetyl-salicylic acid (aspirin) has been used widely to lower the serum uric acid concentration in patients with gout. Therefore, the effect of aspirin on serum urate level and urate excretion was studied. On 7 occasions, daily doses of aspirin of 5 grams or more were administered to 3 patients with hyperuricemia due to gout and to 2 normal subjects. This resulted in a decrease of serum urate concentration of 42 to 75 per cent. This could be accounted for by increased renal excretion. Small doses of aspirin of 2 grams per day were given to the 3 patients with gout on 6 occasions. In all these instances, the serum urate level increased by 37 to 69 per cent. This rise in serum urate level was due to a temporary decrease in excretion. Small amounts of aspirin had only a slight and possibly insignificant effect in raising serum uric acid in normal individuals. One gouty subject was encountered whose serum urate concentration was entirely uninfluenced by the administration of aspirin.

The mechanism of the action of aspirin on renal excretion of urates was studied. It was found not to be a change in the ability of the kidneys to concentrate urates, since the maximum urinary urate concentration, obtained during urine concentration tests, remained unaltered when aspirin was given. Although renal urate clearance is highly variable, the maximum value during a 24-hour period remained inversely proportional to the serum concentration. The administration of alkali along with the aspirin in no way altered the effects described.

Meningococcal Arthritis: A Clinical Study. By WILLIAM W. BECKMAN (by invitation) and MARIAN W. ROPES, Boston, Mass.

Certain features of meningococcal arthritis which set it apart from other types of infectious arthritis were observed. Ten of 34 cases of proved meningococcal infection had articular involvement. This incidence of 29 per cent is considerably higher than has been reported. In every case in which joint involvement occurred, meningococci were grown from the blood stream and in all but one a typical purpuric skin rash was present.

The arthritis was usually asymmetrical and, in general, involved only 1 or 2 of the larger joints. The affected joints were swollen and sometimes limited in motion, but in no case were they red or hot. Pain was slight and at times the patient was not aware of joint disease until it was pointed out on physical examination. This was in distinct contrast to the evidence of inflammation in the synovial fluid where leukocyte counts ranging between 760,000 and 42,000 were found at the time of the first observation. In spite of the purulent effusions, it was exceedingly difficult to cultivate the organism, which was obtained from only 1 aspiration in 1 patient. Fourteen subsequent aspirations during 6 weeks in the same patient yielded purulent fluid from which no organism could be grown by any technic.

Another striking characteristic of this arthritis is its failure to respond to sulfonamide therapy. In every case, the meningitis and septicemia responded rapidly to the drug, but the arthritis persisted, and, indeed, on two occasions developed, after the other signs of meningococcus infection had subsided. Seven cases were treated with sulfonamides alone. The drug was continued until the arthritis, whose average duration was 6 weeks, had subsided. Two cases were treated with both sulfonamides and antimeningococcus serum and the arthritis lasted less than one week in both cases. It is possible that serum may be the method of choice for combatting meningococcal arthritis. It is of interest that none of our patients suffered permanent joint damage.

Plasma Antithromboplastic Activity after Severe Hemorrhage. By L. M. TOCANTINS, Philadelphia, Pa.

In patients who have had severe blood loss for some time, the antithromboplastic activity of the plasma is markedly reduced. This and the frequently coincident thrombocytosis may account for the hypercoagulability of the blood which often follows hemorrhage. Diminution in plasma antithromboplastic activity and thrombocytosis may be important contributory factors in the production of vascular thrombosis. Blood or plasma of low antithromboplastic activity usually clots at the same rate whether placed in plain glass or paraffin coated tubes. The blood of individuals who have had severe hemorrhages, and plasma from which antithromboplastin has been removed by contact with adsorbents, often display the same characteristics. The clot accelerating action of a water wettable surface (e.g., glass) seems

to result, at least partly, from an inactivating effect of the surface contact on the plasma antithromboplastin.

The excess of antithromboplastin responsible for the reduced coagulability of hemophilic blood may also contribute to the exaggeration of the difference between the rate of coagulation of this blood in plain glass tubes as contrasted to paraffin coated tubes.

The Use of Deracil (2-Thio-6-Oxypyrimidine) in the Treatment of Toxic Goiter. By S. L. Gargill and (by invitation) M. F. Lesses, Boston, Mass.

The anti-thyroid effects of Deracil (2-thio-6 oxypyrimidine) have been investigated clinically in 29 patients with toxic diffuse goiter, and 5 patients with toxic nodular goiter. As complicating factors, 1 patient had pernicious anemia in remission; 1 had mitral stenosis with auricular fibrillation; 2 had hypertensive heart disease; and three had paroxysmal auricular fibrillation.

The majority of the patients were initially hospitalized for 2 weeks. During this period, the B.M.R. level before treatment was established, following which Deracil was administered. The B.M.R. was measured at weekly intervals and leukocyte counts were done on alternate days with differential counts if the total leukocyte count fell below 4000 per c. mm.

Deracil was administered in doses of 0.6 to 0.8 gram daily. The dosage was reduced to 0.1 or 0.2 gram daily as normal or subnormal levels of metabolism were attained. All patients received high calorie diets and occasional small doses of sedatives. Digitalis or quinidine sulphate were given when indicated.

Clinical improvement, as measured by the fall in metabolic rate, weight gain, and decreased pulse rate, usually occurred 2 weeks after Deracil was started, and was fairly complete at the end of 1 month. The goiters decreased in size and became softer and eye signs improved.

In 4 patients, there was initial aggravation of symptoms and signs for several days. Four patients developed myxedema which disappeared upon omission of Deracil. Two patients developed bilateral enlargement of the submaxillary glands, accompanied by slight fever. This complication improved within 1 week despite continuation of therapy. Two patients developed drug fever, which disappeared upon omission of Deracil. One patient, a woman aged 57, developed agranulocytosis after taking Deracil, 0.2 gram daily, for 6 weeks. While definitely recovering from the agranulocytosis, she suddenly died from tracheal mucus plug and pulmonary atelectasis.

Deracil is an effective but treacherous antithyroid drug.

Treatment of Toxic Goiter by Irradiation of the Pituitary. By Willard O. Thompson and (by invitation) Phebe K. Thompson, Chicago, Ill.

Ever since the demonstration of the thyrotropic activity of the pituitary there has been great interest in its rôle in toxic goiter. During the past several years, we have observed the effect of irradiation of the pituitary in 38 patients with toxic goiter. In 7 of them, the metabolism dropped permanently to within normal limits. In 16, a temporary reduction in metabolism of from 15 to 52 points was observed. In 15 patients, there was no change. These observations appear to support the hypothesis that in some patients with toxic goiter, the anterior lobe of the pituitary stimulates the thyroid to overactivity. While the results of treatment with thiourea, thiouracil, and radioactive iodine are very important, these materials do not appear to attack the underlying cause of the disease.

An Experimental Attempt to Transmit Primary Atypical Pneumonia in Human Volunteers. By Commission on Acute Respiratory Diseases. (Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, U. S. Army). Presented by Theodore J. Abernethy (introduced by John H. Dingle), Fort Bragg, N. C.

A preliminary experiment has been conducted in human volunteers in an attempt to transmit primary atypical pneumonia. Throat washings and sputa were obtained from 7 hospitalized soldiers manifesting characteristic symptoms, physical signs, and roentgenographic evidence of primary atypical pneumonia. The unfiltered specimens were sprayed into the respiratory tracts of 12 individuals. The volunteers were kept in group isolation for a period of 6 weeks and observed daily for the development of any symptoms and signs of illness.

Respiratory illnesses, varying in clinical manifestations and severity, developed in 10 of the 12 volunteers. In 6 men, symptoms appeared between 5 and 12 days after the first inoculation. Constitutional symptoms of moderate severity were noted by 7 patients. Subcrepitant "sticky" râles were detected in the lungs of 5 patients. Eight volunteers developed fever (99° F. or above) following the inoculations; in 5, the maximum temperature was over 100° F. The roentgenograms of 3 patients revealed definite but minimal pulmonary infiltration. Cold hemagglutinins appeared in the convalescent sera of 3 patients; agglutinins for an indifferent streptococcus (Rockefeller No. 344) did not develop during illness.

Evidence is presented that illnesses closely resembling primary atypical pneumonia developed in certain of those who received the inoculations.

Changes in Vascular Permeability Correlated with the Incidence of Encephalopathy Encountered during the 5-Day Treatment of Syphilis with Mapharsen. By O. Tod Mallery, Jr. (by invitation) and Arthur C. Curtis, Ann Arbor, Mich.

A study of 84 consecutive patients receiving intensive mapharsen therapy for syphilis is reported wherein capillary studies, using both positive and negative pressure tests, were done before and after therapy. The treatment consisted of a total of 1200 mgm. of mapharsen given as a slow intravenous drip over a 5-day period.

A clinical diagnosis of hemorrhagic encephalopathy was encountered in 7 cases and post mortem examination confirmed the diagnosis in 2 fatal instances. This complication occurred in 6 females and 1 male. A positive tourniquet test and a lowered negative pressure reading were found in a majority of the patients at the onset of clinical symptoms. A return to pre-treatment values was observed with recovery.

No significant difference was observed between the pre- and post-therapy whole blood vitamin C levels, plasma prothrombin, bleeding, and clotting times. Platelet counts were likewise unaltered.

The finding of a positive tourniquet test in the pre-treatment group proved to be a warning of an impending reaction in 3 of 6 patients. The use of the positive pressure test is suggested as an aid in selecting and following patients receiving intensive arsenical therapy.

Hypogonadotrophic Eunuchoidism: Its Physiology, Diagnosis, Prognosis, and Treatment. By CARL G. HELLER and WARREN O. NELSON (introduced by Elmer L. Sevringhaus), Detroit, Mich.

Hypogonadism in males may be due to a primary defect of the testis or secondary to failure of the gonadotrophic portion of the anterior pituitary. Distinguishing the latter group from the former is of importance in determining prognosis and therapy. Life-long substitutional therapy is needed by individuals with primary leydig-cell failure whereas stimulatory therapy in these cases of hypogonadotrophic eunuchoids may precipitate spontaneous puberty, in which case no further therapy is necessary.

Thirteen cases of hypogonadotrophic eunuchoidism have been studied clinically and routine laboratory studies have been performed. In addition, biopsies of the testes and mammary glands have been studied histologically. Urinary gonadotrophic excretion, 17-ketosteroid excretion, and estrogen excretion have been determined. Review of the results indicated that administration of chorionic gonadotrophins should stimulate leydig-cell function.

Administration of 1500 I. U. daily caused clinical improvement and biopsies performed during therapy indicated marked leydig-cell stimulation and incomplete stimulation of spermatogenesis. Therefore the administration of a purified preparation of follicle-stimulating hormone was added to the therapeutic regime in some cases while injections of chorionic gonadotrophins were continued. This resulted in additional stimulation of the seminiferous tubules and the production of spermatogenesis, which was proved by study of seminal fluid specimens and of testicular biopsies.

The clinical and laboratory features will be described along with suggestions for a therapeutic test to distinguish this syndrome from other types of eunuchoidism.

*Effect of Methyl Testosterone on Adrenal Cortical and Testicular Precursors of Urinary 17-ketosteroids.** By E. C. REIFENSTEIN, JR. (by invitation), A. P. FORBES (by invitation), F. ALBRIGHT, and E. DONALDSON (by invitation), Boston, Mass.

Since 17-methyl testosterone is not excreted as a 17-ketosteroid, it was employed to study the effect of a testosterone compound on the endogenous production of urinary 17-ketosteroids.

The administration of methyl testosterone decreased the urinary 17-ketosteroids of 5 patients in whom the only source of these substances was the adrenal cortices (2 women with adrenal hyperplasia and Cushing's syndrome, 2 women with adrenal hyperplasia and adrenogenital syndrome, and 1 woman with normal adrenal cortical function) and of 1 patient where the only source was the testes (a male patient with Addison's disease).

Since the production of 17-ketosteroid precursors from both the adrenal cortex and the male gonad is inhibited by the same substance, and if one attributes this inhibition to a decreased production of some pituitary tropic hormone, these findings support the thesis that the same tropic hormone, presumably the lutinizing hormone, stimulates both glands. The 17-ketosteroid excretion by patients with the adreno-genital syndrome was less easily influenced by methyl testosterone than was that of patients with Cushing's syndrome. This is further evidence that the elevated 17-ketosteroid excretion in the former condition is a manifestation of the primary pathology, whereas the increased excretion in the latter condition is an indication of a compensatory process.

The fact that the 17-ketosteroid excretion in the adreno-genital syndrome can be reduced with methyl testosterone gives hope that a non-androgenic steroid may be found which will likewise reduce the 17-ketosteroid excretion in this condition.

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